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THE RELATION OF THRESHOLD OF EXCITABILITY OF NERVE TO CARBON DIOXIDE TENSION

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The relation of excitability of nerve to carbon dioxide tension within physiological limits has not been systematically or graphically examined. The original statement of Waller (1896) that "small amounts of carbon dioxide augment excitability," was based not on observation of threshold for single stimuli but on augmented deflections of a slow-moving galvanometer upon tetanisation of the nerve. Observations on threshold incidental to other work by Borutau and Froehlich (1904), Davis, Pascual and Rice (1928), Necheles and Gerard (1930) and Heinbecker and Bishop (1931), indicated a decrease in excitability. The same result obtained in the recent work of Lehmann (1937).

In the present experiments an effort was made to obtain graphic evidence of the effect of various low tensions of carbon dioxide in moist air on the minimal and maximal action potential threshold of green frog sciatic nerve. The results substantiate a depressant action on excitability by carbon dioxide at all tensions from 1.5 to 180 mm. Hg.

METHOD. The isolated nerve was sealed in a small two-compartment nerve chamber in the usual way. Zinc-zinc sulphate-gelatin electrodes in the one compartment served for leading-off and silver-silver chloride electrodes in the other for stimulating. The level of physiological saline in the bottom of each compartment was raised and lowered at frequent intervals to moisten the nerve by immersion. Air saturated with water vapor passed in a slow stream over the nerve in each compartment at all times except when a carbon dioxide-moist air mixture was substituted in the stimulating compartment. The flow of the carbon dioxide mixture was maintained at a somewhat slower rate than the air passing through the other compartment in order to minimize diffusion along the nerve. The various gas mixtures were conveniently made just before use in small glass flasks over mercury.

The recording apparatus included a one-tube direct current amplifier feeding to a string galvanometer, the string of which was maintained at normal tension. The discharge of a 0.01 mfd. condenser served for stimulation, cathode being nearest the leading-off electrodes. In some experiments a Lapicque type of low resistance shunt was placed across the condenser to minimize alteration of the time constants of the system by possible changes of resistance of nerve with the tension of carbon dioxide. In other experiments a high resistance was put in series with the nerve for the same purpose. After a sufficient number of trials, however, it was clear that a rise of threshold with increasing carbon dioxide tensions was invariably manifested with or without such precautions.

The movement of the recording camera was arranged automatically to vary the charging potential on the condenser from zero to any desired maximum. A rotary type of circuit breaker permitted charge and discharge of the condenser over a wide range of frequency. However, a five per second rate of stimulation was usually maintained. A range of 0–300 millivolts for minimal and 0–1200 or 0–2500 millivolts for maximal threshold determinations was generally adequate except in the preliminary experiments in which a shunt or a high resistance in the stimulating circuit required the use of much higher charging potentials. These had to be avoided, due to incident electrical disturbances, in all of the automatic graphic threshold determinations.

By observing uniformity in making the preparation the initial threshold, visually observed, nearly always lay between 100 and 160 millivolts. Any preparation having a higher threshold was considered unsatisfactory. Immediately after mounting, the threshold was visually determined at minute intervals for ten to fifteen minutes or to apparent stability. A photographic record of responses covering the stimulation range was then taken as normal threshold determination. Thereafter the carbon dioxide-moist air mixture was introduced into the stimulating compartment and the changing threshold visually followed to a new level of stability at which time a second photographic record was taken. The flow of the carbon dioxide mixture was then stopped, moist air substituted, the recovery of threshold followed visually to a final level of stability and a recovery photographic record taken. In this way the effect of gas mixtures ranging from 1.5 to 180 mm. Hg carbon dioxide tension was examined using a fresh nerve preparation for each mixture.

Results. For all tensions of carbon dioxide used the effect of admitting the gas mixture to the stimulating compartment was a progressive depression of excitability. The minimal threshold, visually followed, began to rise in a few seconds and reached a new level of stability in a few minutes. Removal of the gas resulted in almost complete recovery of excitability after several minutes. Typical records made at the normal threshold

level, during exposure to carbon dioxide and upon recovery, are shown in figure 1, a, b, and c.

The heavy diagonal stimulus intensity lines together with a corresponding calibration curve, served to determine the potential to which the

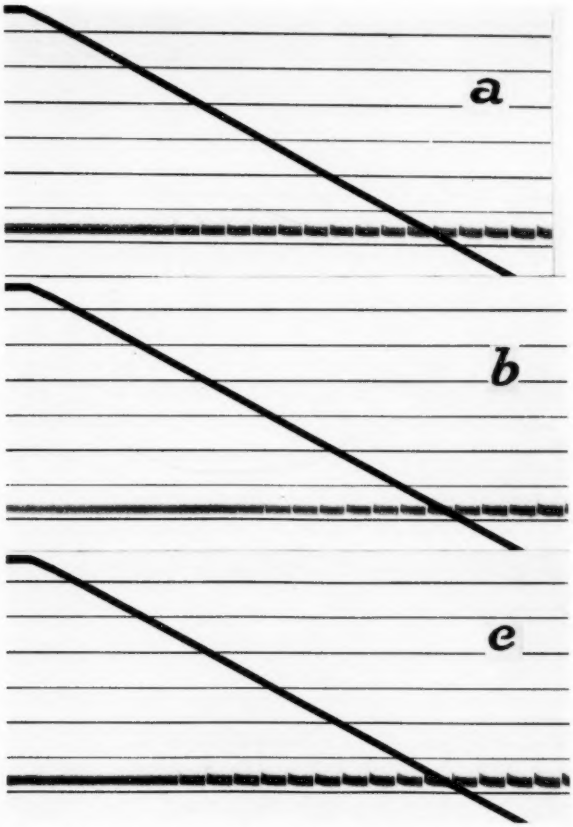

Fig. 1. Effect of CO_2 at 22.3 mm. Hg on the minimal and maximal action potential threshold of frog sciatic nerve. a, normal; b, depression level after 5 minutes' CO_2; c, nearly complete recovery 8 minutes after removal of CO_2.

condenser was charged at threshold points. In the case of figure 1, the minimal threshold was raised 34 per cent and the maximal 40 per cent by exposure to carbon dioxide at 22.3 mm. Hg.

The per cent rise of minimal and maximal thresholds for the series of carbon dioxide tensions employed, is shown in figure 2, a and b. A smooth

directional curve was drawn through the points to emphasize the non-linear relationship apparently existing between carbon dioxide tension and threshold of excitability. For tensions higher than those examined the curves would turn up sharply since it is well known that the threshold of nerve in pure carbon dioxide rises to infinity.

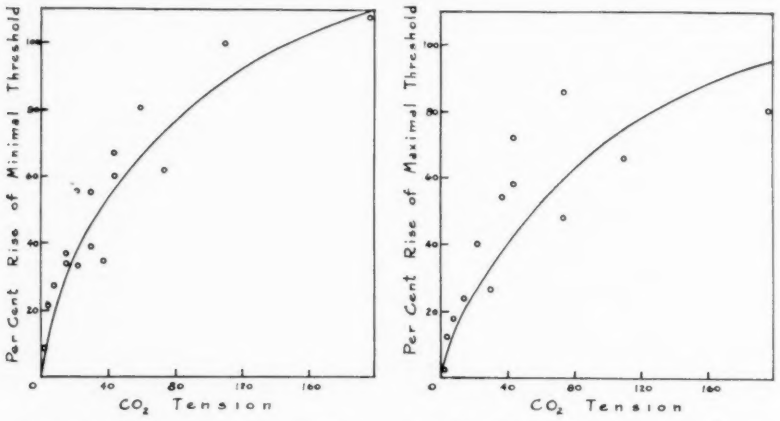

Fig. 2. Relation of excitability of nerve to carbon dioxide tension shown by per cent rise of minimal (a) and maximal (b) threshold.

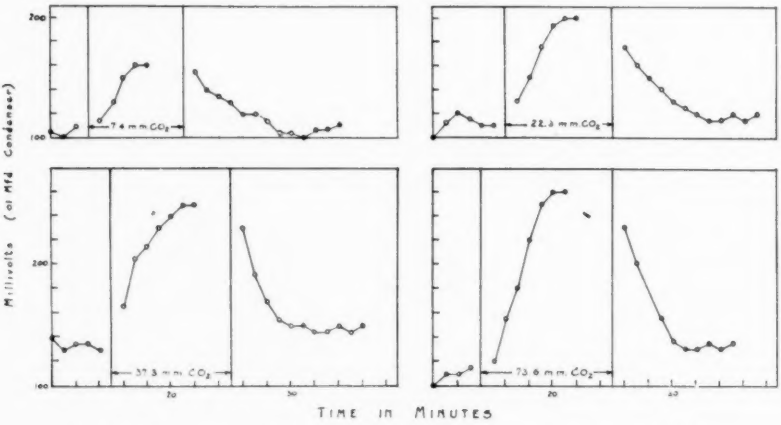

Fig. 3. Time course of minimal threshold at various tensions of CO_2

In the recent work of Lehmann (1937) on the relation of pH to action of mammalian nerve fibers, a nearly linear relationship between change of pH by carbon dioxide and relative threshold of excitation is described. The result may probably be accounted for by the fact that the threshold

determinations were made visually over a narrow range of pH (7.2–7.9). In this connection it may be recalled that a non-linear relationship roughly like that shown in our own figure 2, was reported by Root (1932) as existing between oxygen consumption of nerve and carbon dioxide tension.

From available data on visually determined thresholds a curve could be drawn lying well above those in figure 2, and rising much more rapidly. Obviously the graphic method of threshold determination gives a more reliable picture of the actual relationship of excitability to tension of carbon dioxide. The course of the threshold changes from the time of introducing the gas to the time of recovery is perhaps adequately given by the data obtained in visual observations. In figure 3, time-threshold curves are shown for a series of carbon dioxide tensions.

These curves offer additional evidence that the threshold is raised at all intermediate tensions from zero to any given maximum in the external medium. It would seem highly probable that any increase of carbon dioxide above that which exists in the tissue by its own production results in a depression of excitability.

The time necessary to reach maximum depression at any given tension varied between 4 and 8 minutes, apparently without relationship to the tension. Complete recovery on removal of the gas followed in about the same length of time. In 25 determinations the average time was 6.3 minutes. This is almost exactly the time one can calculate to be required for gas diffusion into tissue 0.8 mm. thick (Hill, 1928), which was approximately the average thickness of the nerves used.

It will be noted in figure 3 that the return to normal is not as complete for the higher tensions of the series. Comparison with untreated control nerves has given the impression that the rate of deterioration of nerve is decidedly increased by exposure to carbon dioxide at tensions over 35 mm. Hg.

Mention may be made of some preliminary experiments in which various weak carbon dioxide mixtures were directed into the leading-off compartment of the nerve chamber. The purpose was to determine the advisability of having the leading-off electrodes outside the portion of nerve subjected to carbon dioxide in the threshold experiments reported above. With care to prevent diffusion along the nerve as much as possible, no significant effect on threshold, as determined outside the leading-off compartment, was observed. The prominent results were an increased amplitude of response and an increase of negative after-potential, the same as previously noted by Davis, Pascual and Rice (1928). However, neither effect bore any obvious relation to the tension of carbon dioxide employed. An optimum tension as described by the authors was not found. The reasons for the increase itself are probably mostly instrumental, as discussed in the paper of Heinbecker and Bishop (1931).

SUMMARY

The relation of excitability of frog sciatic nerve to carbon dioxide tension of the external medium was examined by systematic, graphic action potential threshold determinations. Superiority of the graphic method over visual observation was stressed.

At tensions of carbon dioxide from 1.5 to 180 mm. Hg, the minimal and maximal threshold was invariably raised and reached a maximum which was dependent on the tension.

Graphic evidence was supplemented by visual observation of threshold throughout the course of in- and out-diffusion of the gas thereby confirming the depressant quality of carbon dioxide for any tension above that which exists in the tissue due to its own production.

The time required to reach maximum depressant effect was very nearly that which can be calculated to be required for gas diffusion into the tissue.

Removal of the gas from the external medium resulted in almost complete recovery of excitability for tensions below 35 mm. Hg. Above that tension recovery was never complete and deterioration of the nerve seemed to be accelerated.

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THE EFFECT OF FLUIDS GIVEN INTRAPERITONEALLY, INTRAVENOUSLY AND BY MOUTH ON THE VOLUME OF THORACIC DUCT LYMPH IN DOGS

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The part played by the lymphatics in the absorption of fluid and particulate matter from the abdominal cavity has been a subject of some controversy. Detailed reviews of the literature as published by Bolton (1), Siperstein and Sansby (2) and by Cunningham (3) bear out this fact and reveal as well that the relative importance of the blood vessels and the lymphatics in the quantitative removal of fluid from the peritoneum still is not entirely clear. The problem has been studied chiefly in two ways: 1, by measurements of the appearance time and concentration in thoracic duct lymph of dyes introduced into the peritoneal cavity (4, 5), and 2, by determinations of volume and pressure of thoracic duct lymph and the effect on these of fluids administered intraperitoneally (6, 7).

It has not been made clear, however, by these and other studies what part the lymphatics have in the volumetric removal from the abdominal cavity of large quantities of fluid. The implication has been made that complex fluids containing protein, such as ascitic fluid, are absorbed almost entirely by the lymphatics (1), and on that basis a definite increase in lymph volume might be expected following their intraperitoneal administration. The purpose of this study is to afford further data to show on a quantitative basis how little or how much the lymphatics are concerned with the volumetric removal of simple and complex fluids introduced into the abdomen. Also, to what extent fluids given by other routes affect the flow of lymph from the lymphatic duct. The work was undertaken in connection with experimental studies concerning the general subject of the removal of ascitic fluid.

METHODS AND RESULTS. In all experiments healthy female dogs weighing from 10 to 18 kgm. were used. They were anesthetized with sodium pentobarbital given intravenously in the dose of 35 mgm. per kilogram body weight. With the animal in the supine position the thoracic duct was cannulated at its confluence with the left subclavian vein, the other lymphatic tributaries first having been isolated and tied off. The cannula

was made secure in position by a suture through the skin of the neck so as to afford a free flow of lymph. The lymph was collected throughout the experiment by removing it from the cannula with a fine glass pipette, measurements being made at five-minute intervals. Urine was collected by means of an inlying catheter.

As soon as an even and steady flow of lymph was established by control measurements over a period of 20 to 40 minutes, fluids of different composition were given to the animal by one of three routes; into the peritoneal

TABLE 1

FLUID GIVEN	VOLUME OF FLUID GIVEN	AVERAGE VOLUME OF THORACIC DUCT LYMPH IN 5-MINUTE PERIOD	
		Before giving fluid	After giving fluid
	cc.	cc.	cc.
1. Intraperitoneally			
(a) 0.9% saline	440	1.8 (5)	1.7 (13)
(b) 0.9% saline	880	2.5 (5)	2.6 (15)
(c) 0.9% saline	387	3.7 (14)	2.0 (16)
(d) Distilled water	300	1.9 (3)	1.7 (10)
(e) 6% acacia in 0.9% saline	440	1.7 (13)	1.5 (40)
(f) 6% acacia in 0.9% saline	360	1.6 (6)	1.2 (7)
(g) 6% acacia in 0.9% saline	500	1.7 (15)	3.5 (53)
(h) Horse serum	300	2.6 (15)	2.5 (18)
(i) Heparinized blood	400	3.0 (10)	2.3 (48)
2. By stomach tube			
(a) 0.9% saline	360	1.4 (6)	2.8 (13)
(b) 0.9% saline	500	3.3 (3)	3.8 (9)
(c) Water	500	1.7 (3)	4.1 (13)
(d) Water	500	1.3 (13)	2.3 (12)
(e) Water	500	1.6 (3)	8.0 (8)
3. Intravenously			
(a) 0.9% saline	440	1.5 (40)	3.2 (17)
(b) 0.9% saline	450	1.5 (11)	2.4 (9)

Figures in parenthesis indicate the number of 5-minute periods from which the average is derived.

cavity, by stomach tube, or by vein. The results, according to the method used, are shown in table 1. The table, which gives average figures, does not indicate the rapidity of the changes in the flow of lymph observed in some experiments inasmuch as the increase in flow while considerable lasted only a short time.

1. *Fluid given intraperitoneally.* Volumes of fluid, amounting in most instances to 30 cc. per kilogram of body weight, were introduced into the abdominal cavity through a 16 gauge needle by gravity. From three to seven minutes were required for the injection. This amount was insuffi-

cient to cause appreciable distention of the abdomen or to introduce an element of significant increase in abdominal pressure.

a. Physiological saline (0.9 per cent sodium chloride): Physiological saline was injected intraperitoneally in three dogs. In no instance did there occur any increase in thoracic duct lymph either in the average flow observed for periods of 60 to 80 minutes following injection (table 1) or in the periods immediately following injection.

b. Water: 300 cc. of distilled water were injected intraperitoneally in one instance. There was no change in the flow of thoracic duct lymph during the following 50 minutes. The fluid remaining in the abdominal cavity three hours later contained 1.8 per cent protein and 0.590 per cent sodium chloride.

c. Six per cent acacia in physiological saline: Three dogs received this solution by the peritoneal route. In two there was no appreciable alteration in lymph flow. In the third there occurred a gradual and sustained rise in flow during the last two out of four hours in which measurements were made.

d. Horse serum: To one dog which had shown no increase in lymph flow following the intraperitoneal injection of physiological saline, 300 cc. of horse serum were given by the same route. No change in lymph flow occurred in 90 minutes with this additional fluid, even though a total of 1100 cc. of fluid had been injected within 55 minutes. To determine the presence of horse-serum protein in the thoracic duct lymph, samples of lymph collected at 15-minute intervals were set up against anti-horse rabbit serum. A faintly positive precipitin test was observed in the second specimen (i.e., within 15 to 30 minutes after horse serum was injected) and strongly positive tests in subsequent specimens.

e. Four hundred cubic centimeters of heparinized dog blood were injected into one dog. There was a gradual reduction in volume of thoracic duct lymph during the next four hours. By the end of the first hour the lymph had a definitely pink color and contained 3,050 red blood cells per cubic millimeter, compared to 1,400 in the control specimens. During the second and third hours the red cell count in the lymph rose to 56,000 per cubic millimeter, falling to 46,500 at the end of the fourth hour when the animal was sacrificed. Measurement of the blood remaining in the peritoneal cavity four hours later showed that only 70 cc. had been absorbed, the red cell count on the remaining fluid being 6.5 million cells per cubic millimeter, compared to 6.94 million cells in the blood at the time it was injected. These observations are in keeping with those of Florey and Witts (8).

2. *Fluid given by stomach tube.* In five experiments, 300 to 500 cc. of fluid at body temperature was given by stomach tube:

a. Physiological saline (0.9 per cent sodium chloride): Two dogs re-

ceived saline by this route. In both there was a definite increase in lymph flow appearing within 15 to 30 minutes after introduction of the fluid into the stomach.

b. Water: Water given by stomach tube in three instances produced an even greater increase in lymph flow than that observed after saline. In one animal, fasted for 24 hours before the experiment, the five-minute volume of lymph rose from a control level of 1.6 cc. to 22.0 cc. fifteen minutes after 500 cc. of water had been given (table 1, 2e).

3. *Fluid given intravenously.* Two dogs were given 450 cc. of 0.9 per cent saline intravenously by gravity method. In both cases there was a rise in lymph flow within ten minutes which was sustained for 20 to 30 minutes.

4. *Effect of drugs on lymph flow. Mercupurin.* To three different dogs 1 cc. of mercupurin was given intravenously. In each case the animal had received previously fluid by stomach tube or intraperitoneally. In all three there was a marked decrease in lymph flow within 15 minutes accompanied by good diuretic responses.

Pituitrin. Intravenous pituitrin was given on three occasions in two animals. The first received 1 cc. of surgical pituitrin which evoked a sudden fall in blood pressure and apnea for a minute and also marked intestinal peristalsis and increased salivation. There was a prompt increase in lymph flow during the next five minutes. To another dog two smaller doses of 0.5 cc. were given, each injection being followed by decrease in flow the first five minutes and a slight rise in the next fifteen minutes.

Pilocarpine. Two dogs were given 5 mgm. of pilocarpine intravenously. One showed a 60 per cent increase in lymph flow during the next 30 minutes The other had a 430 per cent increase the following five-minute period when respirations ceased.

5. *Effect of 10 per cent carbon dioxide inhalation.* One animal, whose thoracic duct was cannulated in the usual fashion, had a tracheal cannula inserted which was attached to spirometers containing mixtures of 10 per cent CO_2 and air and 12 per cent CO_2 and air. This dog previously was given 360 cc. of saline and acacia intraperitoneally. The lymph flow being constant, he was switched to the CO_2 mixture. With the rise in the respiratory rate that followed there was an associated increase in lymph flow observed each time the gas mixture was inhaled.

6. *Absorption of fluid from the peritoneal cavity.* No special attempt was made to study the rate of absorption of fluid injected into the abdominal cavity, but in all cases the animals were examined at the close of the experiments and the free fluid measured. In some cases both saline and protein containing material were injected so that the rate of absorption of each was unknown. Our observations showed that the rate of absorption varied greatly from animal to animal, presumably depending on their state of hydration.

In no instance did the volume of lymph collected after fluid was given approximate the amount of fluid absorbed. The greatest absorption occurred in the experiment in which 6 per cent acacia in physiological saline was given (table 1, lg). In this instance the amount of lymph collected equalled 47 per cent of the fluid absorbed. When allowance is made for the expected lymph volume on the basis of collections in the control period, this figure is reduced to 24 per cent. In all other instances the volume of lymph collected was less than that expected from the control measurements indicating that quantitatively this path of absorption was negligible.

7. *The effect of laparotomy on lymph flow.* In two experiments, not included above, the effect of opening the abdomen on thoracic duct lymph flow was observed. There occurred in each instance an appreciable falling off in the volume of lymph immediately after laparotomy. In one instance the reduction of flow was only transient (15 min.), in the other it persisted. In neither instance did sewing up the abdomen have any effect on the subsequent volume of lymph.

Discussion. The results of this study show that the actual volume of fluid removed from the abdominal cavity by the lymphatic route practically is negligible. In only one instance out of nine was there any increase in thoracic duct lymph volume following the administration of intraperitoneal fluid. The increase in this single instance took place very gradually over a period of four and one half hours and occurred in association with the absorption of almost twice the volume of fluid that was absorbed by other animals to whom similar injections had been given. This suggests that the dog may have been in a marked state of dehydration. In all other instances there actually occurred a falling off of lymph volume after giving fluids intraperitoneally. This fact is illuminating in light of the conception held by some that lymphatic absorption is important in the removal of large amounts of intraperitoneal fluid particularly those containing protein. Such a conception has arisen in part because of the fact that particulate matter is so promptly and readily taken up by the abdominal lymphatics. However, the taking up of extremely small quantities of particulate matter, or of proteins, could afford evidence of lymphatic absorption without there being significant removal on a volumetric basis. Our own studies showed that the actual removal of significant amounts of fluid from the peritoneal cavity by way of the lymphatics does not occur. This is in keeping with the opinion expressed by Cunningham, ". . . that solutions which are absorbed from the peritoneal cavity pass in large part directly into the blood stream" (3).

In striking contrast to this were the very marked changes in lymph flow which we observed when fluid was given by stomach tube. In such instances there occurred not only an increased flow of lymph but an in-

crease of considerable magnitude. This fact may be explained by a much greater facility for lymphatic absorption in the bowel than in the peritoneal cavity and also by a richer capillary bed for direct removal into the blood stream. In similar fashion intravenous saline causes a significant rise in the volume of lymph formed, a fact previously well established (9).

The reduction in lymph flow occurring with the diuresis induced by mercupurin suggests a diversion of fluid from areas of lymph drainage to the kidneys via the blood stream. This might be interpreted by some as an "extra renal" action of the diuretic, though there is no reason why the same events could not occur as a result of the direct action of the drug on the kidneys. After the injection of both pituitrin and pilocarpine increased peristalsis occurred to a degree that was audible. This may explain the transient rise in lymph flow that occurred following these drugs. The augmented lymph flow observed with an increased rate and depth of respiration has been explained by Drinker (10) as due to "increase in intra-abdominal pressure during inspiration and suction of lymph into the thorax."

SUMMARY

Fluids (physiological saline, water, 6 per cent acacia, horse serum and blood) introduced into the peritoneal cavity of dogs in amounts varying from 400 to 1000 cc. did not cause an increase in volume of thoracic duct lymph in eight out of nine experiments. In one instance, there was a gradual increase in lymph flow after giving 6 per cent acacia intraperitoneally.

Physiological saline and water given by mouth and saline given intravenously produced a prompt rise in thoracic duct lymph flow.

Diuresis induced by mercupurin was accompanied by marked decrease in lymph flow.

Pituitrin and pilocarpine given intravenously increased the flow of thoracic duct lymph as did the rapid respiratory rate resulting from the inhalation of carbon dioxide.

These studies indicate that lymphatic absorption as measured by the volume of thoracic duct lymph is relatively unimportant in the quantitative removal of fluid from the peritoneal cavity.

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THE UREA CLEARANCE OF CATS WITH DIABETES INSIPIDUS

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The present studies were undertaken to determine whether the urea clearance in cats with surgically induced experimental diabetes is grossly different from that in normal cats, and to study the relation of the urea clearance to urine volume in these animals.

METHODS. In six cats hypothalamic lesions were made through a subtemporal exposure of the base of the brain. The lesions were transverse ones made with the bent tip of a dissecting needle, and were designed to interrupt the supra-optico-hypophyseal system, *1*, at the supra-optic nucleus; *2*, in the ventral part of the tuber cinereum, or *3*, in the pituitary stalk. It may be stated here that at this time we cannot be certain of the exact lesions in our animals as they are all still living.

Fifty-four control urea clearances were done on five normal cats, and sixty-seven on six cats with permanent experimental diabetes insipidus, in order to establish usual levels of renal activity. Once this was accomplished, low rates of urine flow in the polyuric cats were brought about by the intravenous injection of 0.5 cc. of active pitressin, and urea clearances were done both immediately before and after the injection. The same procedure was repeated on normal cats. At a later period, the urine flow in the polyuric cats was elevated above its usual level by the intraperitoneal administrâtion of 6.0 grams of fresh anterior lobe of beef pituitary suspended in saline. About a week after the injection, when the diuretic effect of the anterior pituitary was obvious, urea clearances were done, pitressin was injected, and the urea clearances repeated. The normal cats were subjected to the same procedures and urea clearances obtained. Finally, the normal cats were given water by stomach tube to bring their urine flows up to rates comparable to the diabetic animals and the clearances determined.

Urine specimens were collected from these animals over known periods of time. Often no catheterization was done, for, on a number of occasions, analysis of the bladder washings after voiding revealed insignificant amounts of residual urine. Catheterization was resorted to when the periods were brief and the urine volumes small. Blood samples were ob-

TABLE 1

Urea clearances on cats with diabetes insipidus before and after administration of anterior pituitary and pitressin

CAT	DATE	AVERAGE BLOOD UREA NITROGEN	TIME	URINE VOLUME	CLEARANCE
a. Before administration of pituitary substance					
			minutes	*cc./min.*	*cc./sq. m./min.*
1	1/27	20.4	85	0.144	14.9
			78	0.064	10.7
	2/3	29.8	1172	0.196	10.6
	2/10	21.2	472	0.123	11.9
			275	0.123	11.3
	2/17	23.7	471	0.178	15.5
			325	0.126	11.0
	2/24	16.5	275	0.080	11.0
			137	0.197	16.8
	3/17	19.1	283	0.103	9.6
3	2/3	26.5	32	0.231	7.3
			21	0.209	7.4
			48	0.200	7.9
	2/10	27.2	201	0.109	6.1
	2/17	19.8	62	0.500	20.8
			74	0.527	21.8
			125	0.304	14.2
	2/24	29.4	453	0.125	6.4
			205	0.077	5.5
	3/17	29.3	276	0.091	6.4
	3/22	23.9	382	0.264	13.2
			135	0.119	8.3
5	1/27	20.1	65	0.369	17.7
	2/3	23.4	357	0.195	10.4
			1010	0.279	14.3
	2/10	15.9	186	0.226	12.2
			224	0.214	12.5
	2/17	19.1	214	0.238	14.6
			185	0.259	14.8
	3/3	14.3	81	0.840	25.4
			118	0.398	14.8
			100	0.325	13.8
		14.0	164	0.256	12.8
			102	0.324	13.2
		13.9	107	0.327	14.4
			103	0.485	20.5
			42	0.429	15.9
	3/10	15.3	58	0.966	23.0
			63	0.921	23.2
			110	0.664	19.3

TABLE 1—*Continued*

CAT	DATE	AVERAGE BLOOD UREA NITROGEN	TIME	URINE VOLUME	CLEARANCE
a. Before administration of pituitary substance—*Continued*					
			minutes	*cc./min.*	*cc./sq. m./min.*
			88	0.500	15.8
			71	0.535	16.9
			93	0.452	15.3
	3/17	25.0	115	0.417	16.3
			198	0.247	11.1
			185	0.189	10.8
10	2/3	24.0	229	0.162	9.1
			311	0.125	8.3
	2/10	26.7	241	0.007	5.6
			422	0.107	8.6
	2/17	23.1	297	0.104	8.5
			308	0.120	10.0
	3/3	22.5	214	0.101	7.8
			157	0.147	11.1
	3/10	20.7	307	0.241	13.1
			159	0.164	10.3
11	1/27	18.1	90	0.030	10.7
			10	0.100	12.9
	2/3	18.1	437	0.115	12.4
			974	0.072	14.0
	2/10	24.7	541	0.083	8.7
			145	0.088	13.8
	3/3	21.2	192	0.104	11.1
	3/10	20.0	304	0.072	10.4
			173	0.075	8.9
13	2/24	12.4	406	0.217	27.6
			127	0.276	26.0
Average				0.249	13.1
b. Immediately after administration of pitressin					
1	3/17	18.1	119	0.168	18.2
			119	0.076	16.9
3	3/22	29.3	185	0.054	8.3
5	3/10	15.3	215	0.237	22.2
			64	0.044	14.1
	3/17	23.3	94	0.055	13.2
			124	0.086	19.4
Average				0.103	16.0

TABLE 1—*Concluded*

CAT	DATE	AVERAGE BLOOD UREA NITROGEN	TIME	URINE VOLUME	CLEARANCE
c. After administration of anterior pituitary on 3/17 and 3/18					
			minutes	*cc./min.*	*cc./sq. m./min.*
5	3/22	16.8	91	0.615	27.6
			87	0.517	23.4
			102	0.471	22.2
			209	0.321	15.8
			61	0.426	20.2
10	3/23	12.2	209	0.320	32.3
			188	0.280	27.2
11	3/22	13.5	242	0.155	25.2
13	3/22	13.9	271	0.196	18.3
Average				0.367	23.6
d. Immediately following pitressin after anterior pituitary					
5	3/22	16.8	146	0.092	17.3
			75	0.055	16.0
11	3/22	13.5	280	0.068	20.9
10	3/23	12.6	223	0.067	21.3
			52	0.019	13.5
13	3/22	13.9	268	0.047	18.1
Average				0.061	17.8

tained during the middle of the clearance periods. The blood and urine were analyzed for urea nitrogen by the hypobromite technique of Van Slyke and Kugel (1933). The clearances were calculated according to Møller, McIntosh and Van Slyke (1928) as *cubic centimeters of blood cleared of urea per square meter of body surface per minute.* The time intervals were varied as much as possible to obtain results over long as well as over short periods so that average function, as calculated from a 10-hour clearance, could be compared with the level of function over very short periods at the same time. To eliminate, insofar as possible, physiological variation of renal function, the cats were kept on a constant diet of 50 grams of beef heart and 100 cc. of milk daily throughout the duration of the experiment.

RESULTS. Following the operative procedure, and immediately after recovery from the anesthetic, all the cats had an increased water intake and an augmented excretion of very dilute urine. However, within 72 hours

this disturbance disappeared, and the water exchange remained within normal limits until two to three weeks after the operation. At this time there began an increase in the volume of urine, a decrease in its specific

TABLE 2

Urea clearances on normal cats before and after administration of anterior pituitary, pitressin and water

CAT	DATE	AVERAGE BLOOD UREA NITROGEN	TIME	URINE VOLUME	CLEARANCE
a. Normal control clearances					
			minutes	*cc./min.*	*cc./sq. m./min.*
7	2/10	18.9	558	0.029	10.9
			152	0.020	6.4
	3/17	18.5	420	0.022	14.3
9	2/24	17.4	660	0.017	16.0
	3/10	10.5	104	0.008	10.7
14	2/10	15.0	238	0.017	7.4
			113	0.018	7.8
	2/24	12.9	456	0.011	17.3
	3/10	15.6	77	0.234	13.5
			605	0.014	8.8
	3/17	17.6	520	0.016	14.5
12	2/10	14.8	345	0.058	18.5
			186	0.061	15.5
	2/17	12.3	230	0.048	21.8
			359	0.036	18.8
	3/10	10.0	172	0.039	17.0
			305	0.038	17.6
			212	0.076	16.1
	3/17	17.3	392	0.044	16.1
			114	0.043	16.3
Average				0.042	14.3
b. Immediately after pitressin					
7	3/17	17.8	48	0.050	11.7
			148	0.067	31.1
14	3/17	17.6	60	0.025	8.4
			141	0.064	44.3
12	3/17	17.5	55	0.058	11.9
			152	0.064	24.9
Average				0.055	22.0

TABLE 2—*Concluded*

CAT	DATE	AVERAGE BLOOD UREA NITROGEN	TIME	URINE VOLUME	CLEARANCE
c. After 6.0 grams anterior pituitary given 3/17 and 3/18					
			minutes	*cc./min.*	*cc./sq. m./min.*
7	3/22	11.9	354	0.048	30.0
	3/29	15.1	279	0.048	20.1
			129	0.139	18.5
9	3/22	9.6	421	0.039	28.6
12	3/22	12.4	388	0.057	24.9
			156	0.099	28.6
			169	0.041	14.3
14	3/22	9.5	241	0.033	34.8
			178	0.042	34.8
	3/29	14.3	313	0.037	40.4
			207	0.128	39.1
15	3/22	16.3	398	0.030	23.3
	3/29	17.0	311	0.018	19.0
Average				0.063	27.4
d. Immediately after pitressin given 5 days after anterior pituitary					
7	3/22	11.9	284	0.035	24.9
9	3/22	9.6	316	0.073	27.9
14	3/22	9.5	207	0.034	31.4
15	3/22	16.3	272	0.105	40.3
Average				0.062	31.1
e. Immediately after water given by stomach tube					
7	3/29	15.1	23	0.348	34.6
			37	0.527	34.4
			23	1.065	43.7
			18	1.100	41.5
			32	0.969	36.5
14	3/29	11.9	29	0.914	68.1
			27	0.796	55.5
15	3/29	14.8	128	0.234	35.8
			31	1.161	57.3
			43	0.942	46.2
			46	0.446	27.1
Average				0.773	43.7

gravity, and an increase in water consumption. These alterations in water exchange reached a maximum 24 to 35 days after the intracranial operation, and have persisted more than 9 months. Under the conditions in the animal quarters, normal cats usually drink no water, and never more than 50 cc. per day, and excrete 50 to 125 cc. of urine with a specific gravity ranging from 1.025 to 1.050. The cats with the permanent polyuria daily drink 250 to 1100 cc. of water, and void, within that time, 200 to 1000 cc. of urine having a specific gravity of 1.005 to 1.015.

Our results, shown in some detail in table 1, indicate that there is no apparent difference between normal and diabetic cats insofar as their urea excreting mechanisms are concerned. The average urea clearance of normal cats was 14.3 cc. per sq. m. per min., and of diabetic cats 13.1 cc. per sq. m. per min. The difference is not significant. In the diabetic cats the relationship between minute urine volume and clearance was more apparent than in the normal cat because of the wider range of values.

A considerable increase in diuresis, which was noted about 5 days after administration and which lasted at least two weeks, could be obtained in the polyuric cats by the intraperitoneal injection of a saline suspension of the anterior lobe of the pituitary. Urea clearances done during this period of exaggerated diuresis showed values higher than those obtained during control periods on the diabetic cats. On the other hand, the normal cats were quite refractory to the diuretic action of the pituitary injections, and there was no significant effect on the urine flow in these animals. Even though there was no diuresis, the urea clearances were considerably elevated. Pitressin, which suppressed the diuresis in the cats with diabetes insipidus, had no effect on the volume of urine excreted by the normal cats, but after its administration the urea clearances of both the polyuric and normal cats were increased. Pitressin, given to the diabetic cats after anterior pituitary, suppressed the marked diuresis, and the urea clearance dropped significantly, but when given to normal cats, after similar treatment with anterior pituitary, pitressin failed to alter the urine flow and was followed by a further increase in the clearance. These data are included in table 2. It is to be noted that a diuresis caused by giving water by stomach tube to normal cats increased the urea clearance.

Discussion. The increase in urea clearances following water administration seems to be due solely to an increased urine flow, but the increase in clearances following both pitressin and anterior pituitary cannot be explained on this basis, and the data obtained are insufficient to indicate an explanation.

SUMMARY

1. A persistent polyuria has been produced in cats by surgical intervention in the hypothalamico-hypophyseal complex.

2. There is no significant difference between the urea clearance of normal cats and that of cats with diabetes insipidus. The average clearance for normal cats is 14.3 cc. per sq. m. per min. and for operated animals 13.1 cc. per sq. m. per min.

3. Pitressin acts on animals with this type of diabetes insipidus to reduce the minute urine volume, but it has no action on the normal urine flow of the unoperated cats in this series. The urea clearance in both groups of animals was increased by this substance.

4. Anterior pituitary substance increased the urea clearances of both normal cats and those with diabetes insipidus. This was not solely due to increased urine volumes in the latter group, since the same phenomenon was present in the normal cats with an unchanged rate of urine flow.

5. There is a definite tendency, obvious in cats, both normal and with diabetes insipidus, for the urea clearance to increase with increasing urine flows. This change is, however, much less over the usual physiological ranges of urine flow than it is after administration of anterior pituitary.

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COMPARISON OF TISSUE METABOLISM IN NORMAL, SPAYED, SPAYED-THYROIDECTOMIZED AND HYPOPHYSECTOMIZED FEMALE RATS

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The purpose of this report is to compare the metabolism of isolated liver and kidney of normal, spayed, spayed-thyroidectomized, and hypophysectomized female rats. These data also permit comparisons between findings on isolated tissues and those of other workers who have studied basal metabolism under similar conditions.

All metabolic studies were carried out when the animals were 98 to 130 days old. Their care, breeding and diet have been described (1). They had free access to food and water up to the time that they were sacrificed. Ovariectomy was performed at 60 and thyroidectomy at the age of 90 days. Hypophysectomy was carried out by the parapharyngeal route (24) at 90 to 110 days. Since hypophysectomy results in extremely atrophic and functionally inactive ovaries (24), it was unnecessary to spay these animals to make them comparable to the spayed and spayed-thyroidectomized groups. The normal and spayed rats were 98 to 110, the spayed-thyroidectomized 120 to 130, and the hypophysectomized 105 to 125 days old when sacrificed. Normal females include those in oestrus and dioestrus (29). The spayed group consists of untreated and theelin or amniotin treated animals in which treatment had no effect on liver or renal metabolism. Treated animals had been injected with theelin or amniotin 1, 2 or 4 days before the metabolic studies (3). Since neither theelin nor amniotin affect the metabolism of the liver or kidney of spayed-thyroidectomized or hypophysectomized rats, both treated and untreated animals of these groups have been combined (30).

Tissue respiratory rates and quotients were measured with differential volumeters (28). The Ringer solution contained 0.2 per cent glucose (28). In the summary presented in table 1 only those animals are considered which were free from infections of the middle ears or lungs, or remnants of glands that were supposedly removed. Figure 1 is a scatter chart of the individual observations. Statistical analysis of the 4 series, namely, normal, spayed, spayed-thyroidectomized, and hypophysectomized rats, is presented in the second part of table 1. Here are listed

the mean metabolic differences between the various groups as well as the probable errors of the differences. When the ratio of the difference to its probable error is over 4, it is considered statistically significant. All such differences are italicized.

The following changes are noted:

1. Compared with normal tissue metabolism, spaying or spaying and thyroidectomy lower the respiratory rate of the liver and the R.Q. of the kidney; hypophysectomy in addition to the above effects, also lowers the

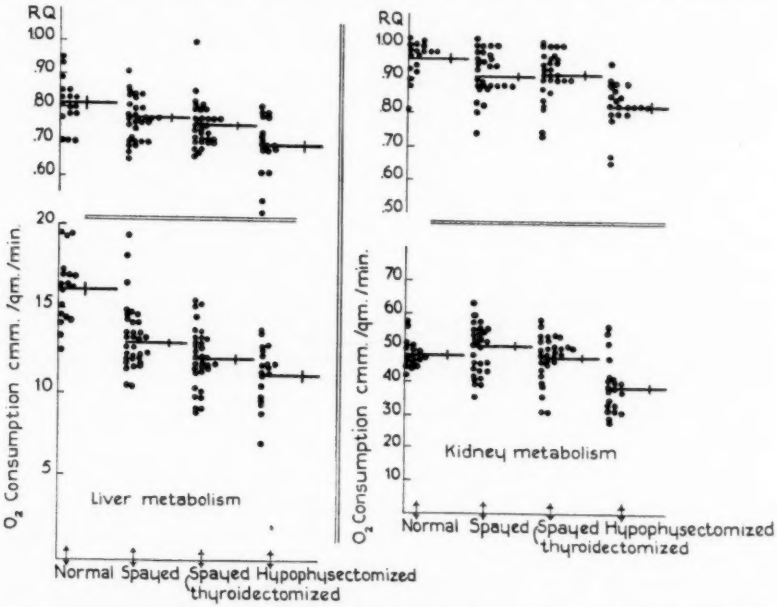

Fig. 1. The respiratory rates and quotients of liver and kidney of normal, spayed, spayed-thyroidectomized and hypophysectomized rats. Horizontal lines = mean values. Vertical lines = 2 × P.E.

R.Q. of the liver, the respiratory rate of the kidney and further lowers the R.Q. of the kidney.

2. Compared with the tissues of spayed rats, those that were spayed and thyroidectomized show that thyroidectomy produces no further statistically significant change in the respiratory rate or quotient of liver and kidney; hypophysectomy, however, significantly lowers the respiratory rates and quotients of both liver and kidney.

3. Hypophysectomized, as compared with spayed-thyroidectomized rats, show statistically significant decreases in liver R.Q. and lower renal respiratory rates and quotients.

Several other interesting findings may be noted. One incompletely hypophysectomized rat, in which 2.1 mgm. of anterior lobe remained, showed no significant alteration from normal unoperated animals in its liver, kidney or anterior pituitary metabolism. The remnant of the pituitary was studied in a respirometer of a type previously described (27). The liver, renal and pituitary respiration had the following normal characteristics of 15.3, 46.5 and 14.9 cmm. O_2 consumption/gm./min. respectively. The R.Q. of the liver and kidney, respectively were 0.72 and 0.90. Four hypophysectomized rats with pyogenic infections, three in the middle ears and one[1] at the operative site had a mean liver respiratory rate

TABLE 1

Liver and kidney metabolism. Comparison of normal, spayed, spayed-thyroidectomized, and hypophysectomized rats

	NO. OF RATS	LIVER				KIDNEY			
		O_2 consumption, cmm./gm./min.		R.Q.		O_2 consumption, cmm./gm./min.		R.Q.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
(1) Normal	18	16.13 ±0.31	1.93	0.810 ±0.014	0.086	48.1 ±0.70	4.31	0.959 ±0.008	0.050
(2) Spayed	25	13.0 ±0.18	1.36	0.773 ±0.010	0.070	50.9 ±0.83	5.82	0.906 ±0.010	0.071
(3) Spayed-thyroidectomized	33	12.12 ±0.22	1.16	0.751 ±0.007	0.061	47.4 ±0.83	6.89	0.913 ±0.008	0.068
(4) Hypophysectomized	21	11.25 ±0.24	1.61	0.690 ±0.013	0.085	38.8 ±1.07	7.32	0.821 ±0.010	0.071

Mean differences and the probable errors of respiratory rates and quotients

Significant differences italicized

COMPARISON OF	DIFF.	PER CENT DIFF.	DIFF. / P.E. DIFF.	DIFF.	DIFF. / P.E. DIFF.	DIFF.	PER CENT DIFF.	DIFF. / P.E. DIFF.	DIFF.	DIFF. / P.E. DIFF.
1-2	*3.13 ±0.36*	19	8.7	0.043 ±0.017	2.5	2.8 ±1.09	6	2.6	*0.053 ±0.013*	4.1
1-3	*4.01 ±0.38*	25	10.6	0.059 ±0.016	3.7	0.7 ±1.09	1	0.6	*0.046 ±0.011*	4.2
1-4	*4.88 ±0.39*	30	12.5	*0.120 ±0.019*	6.3	*9.3 ±1.28*	19	7.3	*0.138 ±0.013*	10.6
2-3	0.88 ±0.28	7	3.1	0.022 ±0.012	1.8	3.5 ±1.17	7	3.0	0.007 ±0.013	0.5
2-4	*1.75 ±0.30*	13	5.8	*0.083 ±0.016*	5.2	*11.1 ±1.35*	22	8.2	*0.082 ±0.014*	5.9
3-4	0.87 ±0.39	7	2.6	*0.061 ±0.015*	4.0	*8.6 ±1.35*	18	6.4	*0.089 ±0.013*	6.9

of 13.7 cmm. O_2/gm./min. and R.Q. of 0.80, both well above the means of the uninfected hypophysectomized rats. These observations confirm previous work (29) in demonstrating the influence of host infection on the metabolism of isolated tissues. They further show that the increase in the respiratory rate and quotient of the liver, which is found in infection, occurs in the absence of the hypophysis.

[1] The liver and spleen of this rat showed numerous areas of focal necrosis with mononuclear cell infiltration, characteristic of paratyphoid. The respiratory rate of the liver was 15.5 cmm. O_2/gm./min., higher than any of 24 observations on uninfected hypophysectomized rats.

Comparison of the changes in basal metabolism of the rat with the metabolism of its isolated tissues after spaying, thyroidectomy or hypophysectomy reveals certain interesting differences. Spaying depresses the respiratory rate of liver about 19 per cent (29), of anterior pituitary 30 per cent (31), of uterus 60 per cent (17), but not of kidney (29). Various observers (4, 15, 26) have found no change in the basal metabolism after gonadectomy. On the other hand, others (6, 8, 18) report about a 20 per cent decrease in basal metabolism.

Thyroidectomy decreases the metabolism of isolated rat diaphragm 20 to 30 per cent (12), skeletal muscle 33 per cent (16), brain 4 per cent (33). It is questionable whether the small change observed in brain tissue is significant. Our own observations on liver and renal respiration in spayed-thyroidectomized rats show a 25 per cent decrease in the liver, as compared with normal, but no change in the kidney. When it is recalled that after spaying the thyroid becomes atrophic (2), it is not surprising that the metabolism of the liver and kidney of the spayed rat is about the same as that of the spayed-thyroidectomized animal. It is generally agreed that thyroidectomy lowers the basal metabolism. According to Smith, Greenwood and Foster (25) this decrease is 27 per cent in the rat. The R.Q. is not influenced by thyroidectomy (7). When compared with normal, spaying lowers the R.Q. of liver and kidney. Thyroidectomy produces no further change in the isolated tissues of spayed rats.

According to Reiss, Hochwald and Druckrey (20) hypophysectomy lowers the respiratory rate of the kidney 28 per cent (5 controls and 11 hypophysectomized) and liver 27 per cent (5 controls and 4 hypophysectomized). The R.Q. of the kidney fell from 0.77 for the controls to 0.65 after hypophysectomy (our own calculations from their data), while that of the liver rose from 0.57 for the controls to 0.67 for the 4 hypophysectomized. The R.Q. was measured by the method of Warburg (32) in which O_2 consumption and CO_2 production were determined on different portions of tissue and no allowance was made for the preformed tissue CO_2. On the other hand, the tables in the report of Franseen and McTiernan (14) show that the livers of hypophysectomized rats had a 50 per cent greater respiratory rate than normal, while the mean R.Q. was 0.67 (6 determinations) as compared with 0.53 (3 determinations) for the normal. They likewise used the method of Warburg. Our own results show that hypophysectomy decreases the liver respiratory rate 30 per cent and the kidney about 20 per cent. The R.Q. of both these tissues is also depressed. It has been found (9) that hypophysectomized-depancreatectomized dogs also show a depressed renal R.Q. as compared with normal. Furthermore, the addition of glucose in the substrate has no influence on the R.Q., while the R.Q. of normal renal tissue is increased by glucose (21). Our findings with regard to incomplete hypophysectomy

confirm those of Reiss, Hochwald and Druckrey (20), and Riddle et al. (22), the latter studying the pigeon, in that incomplete hypophysectomy produces only slight changes in isolated tissue metabolism or in basal metabolism.

The basal metabolism is lowered 33 to 35 per cent by hypophysectomy (13, 25). The depressed metabolism found in isolated tissues confirms the reports of these workers in showing that hypophysectomy lowers the metabolism of isolated tissues more than thyroidectomy. Observations on the basal R.Q. of hypophysectomized fasting rats suggest that there is a possible increase (10) from 0.72 to 0.75 (19 observations in each group). If this increase is significant it is presumably due to increased oxidation of carbohydrate, since the tissues of hypophysectomized fasting rats lose carbohydrate at a greater rate than normal (23). It has been found that hypophysectomized rats form less liver and muscle glycogen from ingested carbohydrate than normal (5, 19). The decreased liver and renal R.Q. observed in our own experiments are difficult to interpret in the light of the above findings. It may be that the liver and kidney carbohydrate combustion is diminished, while that of muscle and other tissues is increased, or else there is an essential difference in our experiments and those reported by other investigators. The tissues in our experiments were derived from non-fasting animals on a normal diet (1). The R.Q. of fed hypophysectomized rats may bear a different relation to the normal than that of fasted ones. The age of our animals at the time of hypophysectomy was 90 days. The age of the animals in Fisher and Pencharz experiments (10) is not stated. In the observations of Fisher, Russell and Cori (11) the rats were 20 to 25 days old at the time of hypophysectomy and were studied at an interval after the operation similar to ours, namely, 15 to 25 days. Since hypophysectomy in young animals results in no weight loss, but diminished growth, while in mature animals there is first rapid, but later slower and continuous, weight loss (24), the difference in metabolism observed in these experiments may be explained. The weight loss is obvious in the depletion of fat stores, especially the subcutaneous tissues of our animals. The lower R.Q. in liver and kidney after hypophysectomy may be due to the oxidation of fats involved in the depletion of fat depots.

SUMMARY AND CONCLUSIONS

Comparisons were made of liver and renal cortex metabolism of normal (oestrus and dioestrus), spayed, spayed-thyroidectomized, and hypophysectomized female rats, 98 to 130 days old. The respiratory rates and quotients of tissues of fed rats were observed in the presence of glucose.

Compared with normal, liver respiration is decreased 19 per cent by spaying, 25 per cent by spaying and thyroidectomy, and 30 per cent by hypophysectomy. The respiratory rate of kidney tissue is not influenced by spaying or spaying and thyroidectomy, but hypophysectomy lowers it

about 20 per cent. The changes in liver metabolism parallel those in basal metabolism under these conditions. The renal metabolic rate is depressed only after hypophysectomy.

The R.Q. of liver and kidney are equally lowered by spaying and spaying and thyroidectomy. Hypophysectomy lowers the R.Q. of liver and kidney even further.

Infection (in hypophysectomized rats) increases the rate of oxygen consumption and the R.Q. of liver in hypophysectomized, as well as in normal or spayed animals.

These observations on the respiratory rates of the tissues of hypophysectomized rats confirm the findings on basal metabolism (13, 25) but not the changes in basal R.Q. (10). An explanation for this inconsistency is suggested.

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WORK-PERFORMANCE OF HYPOPHYSECTOMIZED RATS TREATED WITH CORTIN

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After the removal of the anterior lobe of the pituitary body of the rat the cortex of the adrenal gland undergoes extensive atrophy. It has been shown by Ingle, Hales and Haslerud that large amounts of adrenal cortical tissue are required for maintenance of normal capacity for work of the rat. Ingle (3) has demonstrated that large amounts of cortin must be administered to the adrenalectomized rat to maintain a nearly normal capacity for work. It is logical to expect that the capacity for work of the hypophysectomized rat would be limited by the deficiency in the secretory activity of the atrophic adrenal cortex. In the experiments reported here, hypophysectomized rats that had been treated with cortin were compared with untreated animals in respect to the capacity of the gastrocnemius muscle to sustain work-output when faradic stimulation was applied.

METHODS. Male rats of the Wistar strain, which were uniform as to age and which weighed between 180 and 190 grams each were used in these experiments. The pituitary body was completely removed by the usual, ventral, parapharyngeal approach. Those animals which suffered from either hemorrhage or dyspnea as a result of operation, and the animals concerning which there was any doubt regarding completeness of operation, were discarded immediately. The methods used in the work-tests have been described (2, 3). The initial rate of work was recorded and the animals were observed at appropriate intervals until the stimulated muscle lost responsiveness. To those animals which worked for longer than twenty-four hours, 5 cc. of distilled water were administered subcutaneously at intervals of twelve hours.

The cortin used in these experiments had been assayed previously by the Ingle (3) rat test. The optimal effect on the work-performance of adrenalectomized rats was obtained with 0.5 cc. of the solution administered at each interval of twelve hours.

EXPERIMENTS AND RESULTS. In experiment 1 the work-performance of thirty hypophysectomized rats was studied immediately after the

operation. Fifteen animals of this group were treated with cortin. Each animal received 1 cc. at the beginning of the work-test and at the end of each subsequent period of twelve hours. The remaining fifteen animals did not receive treatment. The initial rates of work were approximately equal for rats of the two groups but the average total amount of work done by the rats which were treated with cortin was greater than that done by the untreated rats. There was overlapping of the performance of individual animals of the two groups to the extent that the difference in averages might have been owing to chance.

In experiment 2 the work-performance of thirty hypophysectomized rats was studied after a delay of four days following operation. Fifteen animals each received 2 cc. of cortin daily in their drinking water. At the beginning of the work-test each animal received 1 cc. of cortin by sub-

TABLE 1

Averages of initial rates of work and weight changes

DESIGNATION	RATS	DAYS DELAY*	POSTOPERATIVE CHANGES IN WEIGHT, GRAMS		INITIAL RATE OF WORK†		COMBINED WEIGHTS OF ADRENALS, MGM.	
			Average	Range	Average	Range	Average	Range
Untreated	15	0			17.9	15–20	29.3	26–38
Cortin treated	15	0			17.0	15–18	28.0	26–32
Untreated	15	4	−30.4	−20 to −29	11.1	9–14	18.5	13–24
Cortin treated	15	4	−39.0	−30 to −48	14.4	13–18	17.8	13–22
Untreated	15	7	−41.1	−30 to −56	8.9	7–11	15.2	10–20
Cortin treated	15	7	−38.5	−28 to −46	15.1	11–19	14.7	12–17

* Between operation and observations.

† Expressed as recorder revolutions per minute. Each recorder revolution approximates 400 gram-centimeters of work.

cutaneous injection and a similar amount, also by injection, at the end of each subsequent period of twelve hours. The remaining fifteen animals were not treated. The initial rates of work and the work-totals of the animals treated with cortin were significantly greater than were those of the untreated animals.

In experiment 3, the work-performance of fifteen rats which were treated with cortin, and of fifteen untreated rats was studied after a delay of seven days and under the conditions of experiment 2. The initial rates of work and the work-totals for the animals treated with cortin were clearly superior to those of the untreated animals.

The group averages and the individual values for total work are summarized in figure 1. Other data are summarized in table 1. In a previous experiment (4) the average work-performance of normal rats of compa-

rable body weight was found to be 67711 recorder revolutions. This value is far greater than that representing any of the performances of the individual hypophysectomized animals studied in the present experiment.

COMMENT. These observations confirm the results of a previous experiment (4) in showing that the work-capacity of the rat is decreased within a few hours following hypophysectomy and that it is decreased further as the interval between the operation and the beginning of the work-tests is increased. The work-performance of the animals which were treated with cortin during periods of four and seven days between the operation and the work-tests was clearly superior to the performance of untreated animals. Although many claims have been made in the literature that the work-performance of the normal animal is increased by administration of cortin, I never have been able to confirm these claims in studies on the rat (unpublished). The results of the present experi-

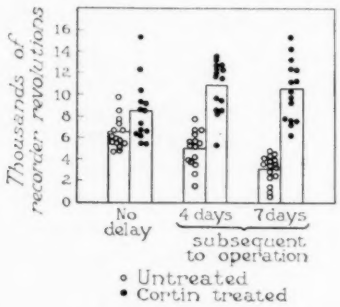

Fig. 1. Group averages and individual records of work performance of hypophysectomized rats.

ment can be explained best by assuming that the adrenal cortex of the hypophysectomized rat is deficient in secretory activity as compared to that of the normal rat and that treatment of the hypophysectomized rat with cortin prevents the physiologic changes which otherwise would result from an inadequate supply of cortin. It is significant that administration of cortin to the hypophysectomized rat did not influence the extent of atrophy of the adrenal glands.

The work-output of the hypophysectomized rat that has been treated with cortin is still very small when compared to the standards for normal animals. This persisting deficiency can be attributed to the numerous other physiologic changes which develop in the hypophysectomized animal from causes other than adrenal cortical insufficiency.

These results have been anticipated by the study of Atwell, who demonstrated that the voluntary activity of the hypophysectomized rat is increased after treatment with cortin. Perla has observed that the resis-

tance of the hypophysectomized rat to histamine is greatly increased by treatment with cortin.

SUMMARY

The work-performance of the hypophysectomized rat that has been treated with cortin is clearly superior to that of untreated animals when the comparisons are made after periods of delay of four and seven days following operation. The initial rates of work were much higher among the animals that had been treated with cortin than among the untreated animals. The work-performance of all hypophysectomized animals studied in these experiments was greatly reduced below the standards for performance of normal animals.

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ISOLATION OF THE CAROTID SINUS PRESSORECEPTIVE RESPIRATORY REFLEX[1]

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A careful review of the literature leads one to believe that the carotid-sinus-stretch respiratory reflex has not been adequately isolated nor rigorously shown to exist. Inconsistent results of various forms of mechanical and electrical irritation of the sinus region and "sinus nerve" have been interpreted as demonstrating the existence of the reflex, but the possibility of pain influences and carotid gland effects question such conclusions. It is true that the use of internal fluid pressure, the natural stimulus for the carotid sinus, has yielded mainly an inverse relationship between changes in intracarotid pressure and breathing, and more consistently when the aortic or vago-aortic nerves were severed than when intact. But even with the use of the natural stimulus and elimination of the antagonistic (normally vicarious) aortic reflex, absolute constancy is lacking (Koch and Mark, 1931; Winder, 1937a; exceptions emphasized by Danielopolu and Marcu, 1931, 1933, and Danielopolu, Marcu and Proca, 1932, may not be real), and several circumstances have seriously complicated all such experiments:

First, the question has been raised as to whether the respiratory effect of carotid occlusion and deocclusion might be due in major part to *altered blood-flow through the carotid gland* (Euler and Liljestrand, 1936; Stella, 1936). Since in vascular "isolation" of the "sinus" the carotid gland arterial supply is ordinarily left patent, the same alternative interpretation may apply to results of the many perfusion experiments purporting to demonstrate a sinus stretch reflex. Actual consequences of this complication are considered later. *Second*, since the gland blood-vessels are very freely anastomosed with the vertebral arteries (Winder, 1933), when intracarotid perfusion pressure excedes anastomotic recurrent pressure, *recurrent circulation of the gland with the animal's own blood may be displaced by fluid from the "isolated" carotid perfusion circuit and vice versa.* Figure 1 shows the case of a routine carotid perfusion experiment in which it appears that this condition could be deliberately brought into evidence.

[1] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

During A the animal was breathing air slightly deficient in O_2. Initially the arterial pressure was so much greater than the intracarotid perfusion pressure that the carotid gland was probably supplied recurrently with the animal's own blood. Increasing the intracarotid pressure reflexly depressed the arterial pressure to below the new intracarotid pressure. The attendant depression of breathing might have resulted from displacement of the animal's own anoxic blood in the carotid gland by less excitatory perfusion fluid, as well as from sinus stretch. During B the animal was breathing O_2-enriched air. A rise in intracarotid pressure this time excited respiration. The displacement of the normal blood in the carotid gland by a now relatively more excitatory perfusion fluid, overcame whatever tendency for sinus-stretch respiratory depression that might have existed.[2] Figure 1 of Gollwitzer-Meier and Schulte (1931) appears to be a similar case. *Third, uncontrolled systemic blood-pressure* may secondarily modify breathing. Figure 2 is an example, where in the

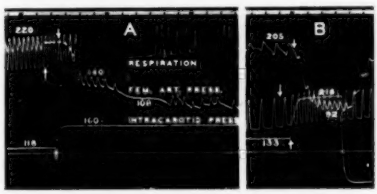

Fig. 1. Dog. Morphine and urethane. "Vagotomized." Uncontrolled pulmonary ventilation and aortic pressure, carotid gland circulation patent. Previous method (Winder, 1937a). Oxygen-deficient air breathed during A and oxygen-enriched air during B.

"vagotomized" dog successive increments of the intracarotid pressure produced only slight changes in breathing until the systemic pressure fell to 76 mm. Hg and lower. At this point increasing periodicity and polypnea appear, which may represent a combination of the influences of carotid reflex inhibition of breathing and of central anemia. We reported occasional augmentation of breathing in vagotomized dogs on raising intracarotid pressure, when the systemic pressure fell to very low levels (Winder, 1937a). *Fourth, uncontrolled pulmonary ventilation* secondarily antagonizes the reflex through the systemic blood composition.

METHOD FINALLY ADOPTED. The animals (dogs) were *just*-adequately[3] anesthetized with morphine (subcutaneously) and urethane (intramuscularly). After painstaking ligation of all efferent vessels of the carotid

[2] It should be noted that the vasomotor stretch reflex, probably operating more exclusively in its domain, appears relatively unaffected by this carotid gland influence.

[3] Urethane in excess abolishes the respiratory reflex.

accessible without great risk of damage to nerve fibers, the remaining fine vessels, including the carotid gland blood-supply, were embolized with lycopodium suspension (Heymans and Bouckaert, 1933). The gland was thus eliminated from any changing influences of carotid blood. The influence of changing pulmonary ventilation was eliminated by use of constant artificial ventilation. The inflation pump was connected with rebreathing tanks and recording spirometer, and adjusted to maintain an apparently normal breathing effort at normal carotid pressure. Mechanical freedom of pulmonary inflation and respiratory movements was afforded by unilateral open pneumothorax and ruptured mediastinum. By means of an adjustable resistance in the expiratory line, the passive

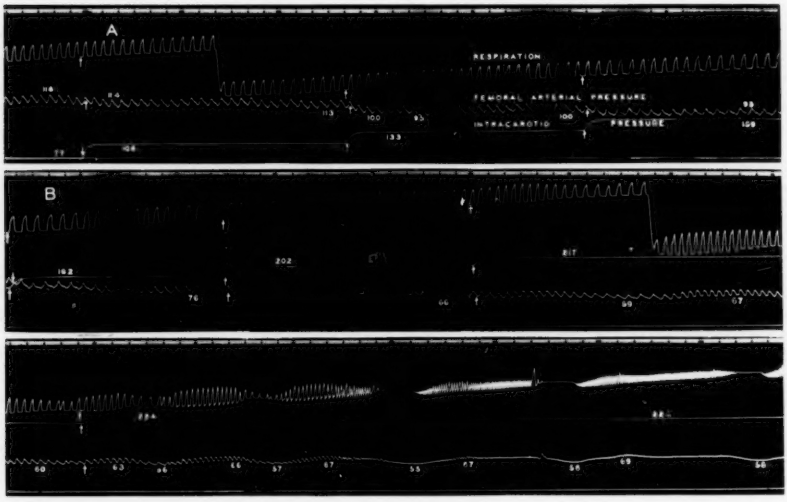

Fig. 2. Dog. Morphine and urethane. "Vagotomized." Uncontrolled pulmonary ventilation and aortic pressure. Previous method (Winder, 1937a).

elastic deflation could be regulated to maintain residual air in the lungs. The aortic pressure was controlled and carotid segment perfused, *with blood of the same composition as that in the animal.* For details of this arrangement see figure 3 and legend. To eliminate unnatural tug on the carotid segment, which preliminary experiments demonstrated to be very important, the positions of the tubes connected with inflow (common carotid) and outflow (lingual artery) cannulae were adjusted by special universal supports. Breathing movements were recorded volumetrically by means of a complete body-plethysmograph connected with recording spirometer and amplifying pulley. The open pneumothorax was connected by means of a cannula and tubing to the exterior. Although the

aortic-pressure compensator and constant pulmonary ventilation would have made aortic denervation unnecessary, the vago-sympathetic-aortic trunks were tied and cut to eliminate any venogenic or pulmonary proprioceptive reflex over the vagi and any carotid reflex changes in cerebral circulation via the cervical sympathetics.

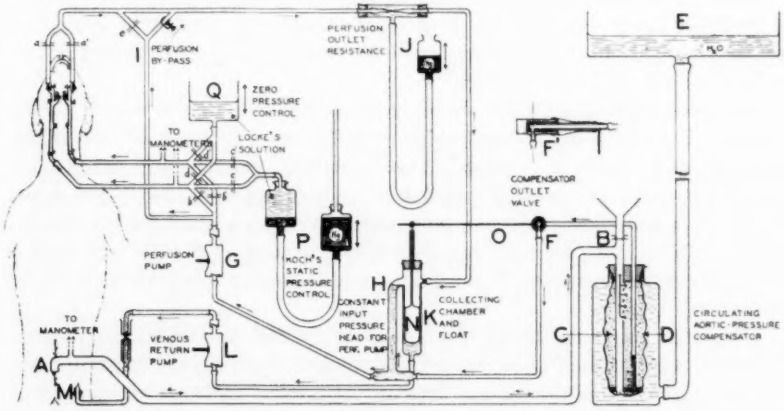

Fig. 3. Carotid segment connections and circulating aortic-pressure compensator.

A to *B*, large-calibre, rigid connection between abdominal aorta and pressure compensator; *C*, circulating blood reserve for pressure compensation, within non-resistant, collapsible, separating bag *D*; *E*, adjustable water pressure-head applied through *D*, C, *B* and *A* to abdominal aorta; *F*, *F'*, automatic compensator-circulation outlet-valve, supplying inlet to carotid perfusion pump *G*, with a constant, overflowing pressure-head *H*; *I*, optional adjustable perfusion by-pass; *J*, control for carotid perfusion outlet resistance; *K*, collecting chamber for perfusion outflow and overflow from pressure head *H*; *L*, venous return pump; *M*, femoral vein connection. By setting the output of venous return pump *L* slightly greater than that of perfusion pump *G*, the float *N* so operates compensator outlet valve *F* through lever *O*, as to insure an overflow from pressure head *H*, and the constant presence of blood in collecting chamber *K* for return pump *L*. By proper manipulation of clamps *a*, *a'*, *b*, *b'*, *c*, *c'*, *d*, *d'*, and *e*, either or both carotid perfusions can be by-passed and either Koch's (1931) static pressure control, *P*, or the zero-pressure control *Q*, connected with either or both carotids. Abdominal aortic and each carotid pressure are recorded by respective mercury manometers. The experimental animal is heparinized. The circuit is supplied with heparinized whole blood from a large donor dog. A constant temperature of 37.5°C. is maintained by a circulating electrically controlled water bath and insulated or water-jacketed connections with the animal.

Four types of experimental sequence were used: in two, *pulsating* endosinual mean perfusion pressures were employed. In one of these the pressure was changed in a *rectangular* fashion from and back to zero for each endosinual pressure tried; in the other it was changed in a progressive

stepwise manner. In two other types of sequence non-pulsatile *static* pressures were used, controlled by means of Koch's device (see fig. 3 and legend). In one of these *rectangular* changes, and in the other *stepwise* changes were used. The pressure was left at any one level until further obvious alteration in breathing movements due to the pressure change had ceased. The records was analyzed at the ends of these intervals. When a series of static pressures were used, the sinus was protected from the blood-substitute in the pressure device by trapping a long column of blood in the carotid and its inlet tube.

RESULTS. Valid data are shown graphically in figures 4 and 5. The changes in rate, tidal volume, and total (minute) volume of breathing movements in terms of percent of the "standard" breathing at zero endosinual pressure, are plotted against corresponding endosinual pressures (mean pulsatile, or static). An interpolated value was used for the standard breathing, intermediate between those measured during zero endosinual pressures immediately preceding, and following recovery from, each "pressure" trial.

When the unstable intricacy of respiratory control is considered, the degree of scattering of points along the smoothed curves is not surprising in spite of the rather elaborate attempt at control in these experiments. Due to the time interval required for attainment of a steady condition at each pressure level the number of points used for individual curves is undesirably small, and doubtless together with scattering has altered the details of curve form in at least some cases. Nevertheless there is an unquestionable qualitative uniformity among the *minute-volume curves*. Rectangular or stepwise, pulsatile or static pressure changes appear to have made no obvious *qualitative* difference among the curves. (But see McCrea and Wiggers, 1933.)

An efficient interaction of the rate and depth components of breathing, even without vagal control, is emphasized by the qualitative uniformity among minute-volume curves existing in spite of a lack of such uniformity among the majority of rate and tidal volume curves.

These minute-volume curves may be considered as activity curves for the carotid sinus pressoreceptive respiratory reflex. Supposing that interpolation over the individual curves is justified when the data so arrived at is treated in class groups, and knowing, as we do from electrical studies, that activation of carotid sinus pressoreceptors varies in a positive sense with endosinual pressure, the curves are interpreted as follows: there is a *threshold pressure-stimulus* below which the reflex is not active. The activity, which is manifested as a *central inhibition*, at first increases slowly per unit increase in pressure-stimulus above threshold, and then more and more rapidly, so that the first part of the curve rises concave to the axis of abscissas. It then reaches a maximum slope or *turning-point* beyond

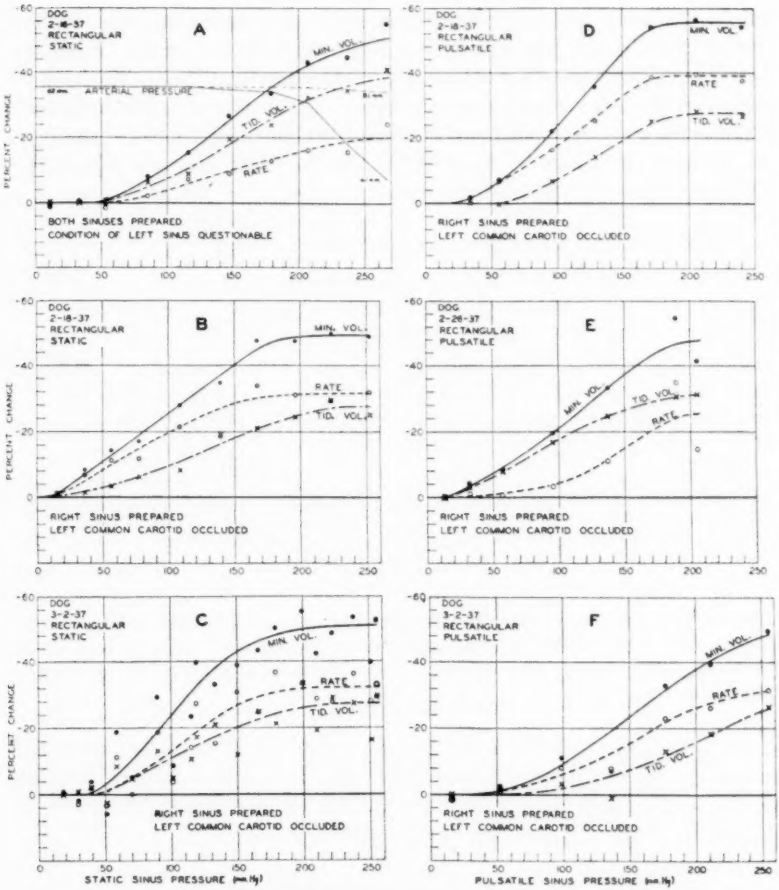

Fig. 4. Successive rectangular pressure changes from and back to zero. Dogs. Morphine and urethane. "Vagotomized." Pulmonary ventilation and aortic pressure controlled. Carotid gland embolized. Open pneumothorax. Breathing movements recorded plethysmographically.

During series *A* there was insufficient reserve blood in the arterial pressure compensator, so that during the latter part of the series arterial pressure was largely under control of the carotid reflex. This fault begins where the light dashed line leaves the light solid line marked "arterial pressure." The latter indicates the standard level corresponding to zero endosinual pressure, the former the level during raised endosinual pressure. The latter part of the respiration curves in this series may be distorted by such lack of control. Graph *C* is in reality plotted from two successive series of observations, one with ascending and the other with descending static endosinual pressures (rectangular). There was an interval of perfusion between the two series.

which it progresses convex to the pressure axis, indicating less and less increase in activity per unit rise in pressure, until the curve finally approaches asymptotically a *maximal pressure-stimulus* level. The general sigmoid curve-form is not unique in physiology, but the striking conformity of qualitative details with those of similar curves constructed for the carotid sinus vascular and cardiac reflexes (Koch, 1931; Lim and Hsu, 1931; Schneyer, 1934, 1935) is peculiarly interesting.

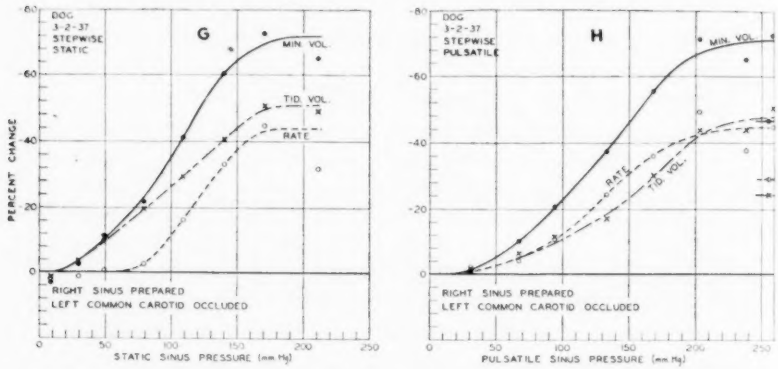

Fig. 5. Progressive stepwise pressure changes. Dog. Morphine and urethane. "Vagotomized." Pulmonary ventilation and aortic pressure controlled. Carotid gland embolized. Open pneumothorax. Breathing movements recorded plethysmographically.

The threshold-pressures and turning-points can be estimated with fair accuracy; the maximal-pressures only roughly because of a commonly greater scattering of data in that region and the asymptotic nature of its approach. These estimations are tabulated below.

CURVE	THRESHOLD-PRESSURE	TURNING-POINT PRESSURE	MAXIMAL-PRESSURE
A	45	150	>270
B	10	95	230
C	35	100	270
D	20	125	210
E	15	125	220
F	35	140	>270
G	15	115	205
H	20	140	260
Median	20	125	245
Arithmetical mean	24	124	
Geometric mean	22	122	

The average threshold-pressure is 20–24 mm. Hg, the average turning-point occurs at 122–125 mm. Hg, and the median maximal-pressure is estimated roughly at 245 mm. Hg. While it is recognized that the degree of relative dispersion is great in these data, the averages become more significant when compared with similar values obtained for the more easily studied vascular and cardiac reflexes. For the dog, threshold pressures of 40 to 60 mm. Hg, turning-point pressures of 103 to 135 mm. Hg with a mode of 120, and maximal-pressures of 190 to >250 mm. Hg are reported (Koch, 1931; Lim and Hsu, 1931; Schneyer, 1934, 1935). Our averages for the respiratory reflex diverge from these values only in the case of the threshold-pressure. This divergence holds for the fundamentally more simple cases of *static* pressures (curves A, B, C and G) as well as for those of pulsatile pressures. While the entire ranges of dispersion scarcely overlap, thus suggesting a possible significance in the divergence, we prefer not to emphasize that. However, we cannot confirm Schneyer's (1935) statement that the threshold-pressure for the respiratory reflex tends to lie *higher* than that for the cardiovascular reflexes.

By use of *rectangular* pressure changes (curves A, B, C, D, E, and F), the *apparent* maximal inhibition of respiratory movements by a single carotid sinus was approximately 50 per cent. But curves G and H, both established by means of progressive *stepwise* series of pressure increases, one with pulsatile and the other with static pressures in the same animal, indicate a maximal inhibition of over 70 per cent, although in this animal *rectangular* series (curves C and F) indicated the usual approximate 50 percent inhibition. Series C was established before either G or H so that this difference is not attributable to deterioration of the preparation. The greater maximal effect with the stepwise procedure is real and readily understood. Although ample time was given at each pressure level in the rectangular series for arrival of respiratory movements at an *apparently* stable level (54–250 sec.), there must have been a continued, very gradual respiratory depression following the primary obvious one, which would have been apparent with time. In the case of progressive, stepwise changes in stimulus, without intermediate reductions of the activity to zero, this secondary gradual depression was allowed opportunity to develop. To test this almost necessary assumption experimentally, the endosinual pressure was raised rectilinearly to some level and left there for many minutes. Figure 6 is an example taken from the same animal with which curves C, F, G, and H were established. Respiratory activity had apparently come to a stable level 2.5 minutes after the static endosinual pressure had been raised from near zero to 255 mm. Hg, approximately the maximal pressure. In accord with the maximal inhibition—which would have been measured at such a time-interval—observed by the rectangular procedure in this and the other animals, the depression of breathing move-

ments was 54 per cent. At the end of 5 minutes, however, it had progressed to 63 per cent, at 10 minutes to 68 per cent, and at 15 minutes to 74 per cent. Recovery at near-zero pressure was complete and less gradual. Thus, somewhat less than the time required for a stepwise series, from zero to maximal, was required for a single rectilinear rise in pressure, from zero to maximal, to develop sensibly the same magnitude of effect.

What underlies the delayed action? The involvement of rate as well as force of breathing bespeaks a "centrally" acting influence. A gradual augmentation of receptor discharge seems improbable. The evidence is against the gradual influence of any carotid reflex change in cerebral circulation great enough to influence breathing, especially in the absence of the cervical sympathetics (Bouckaert and Heymans, 1933, 1935; Schmidt and Pierson, 1934; Schmidt, 1934, 1936; Bouckaert and Jourdan, 1936).

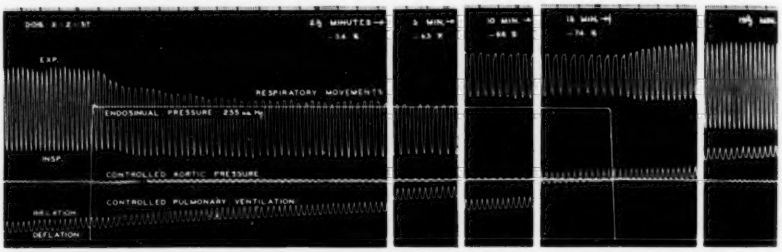

Fig. 6. Dog. Morphine and urethane. "Vagotomized." Pulmonary ventilation and aortic pressure controlled. Carotid gland embolized. Open pneumothorax. Breathing movements recorded plethysmographically. Static endosinual pressures.

An increased velocity of total circulation due to the generalized carotid reflex vasodilatation during constant ventilation, would be expected transiently to cause less efficient pulmonary equilibration of blood which had already passed through the tissues, with consequent secondary respiratory excitation; unless, perchance, the opposite intact carotid gland should participate actively in the reflex dilatation to the extent of an increased irrigation, hence decreased excitation that would decrease breathing more than the diminution in gaseous diffusion gradients at the medulla would increase it. Very soon, however, the improved general circulation might lead to a less excitatory blood (Gesell, Krueger, Gorham and Bernthal, 1930). Decreasing metabolism associated with decreasing respiratory efforts must lead gradually, during constant pulmonary ventilation, to a less excitatory blood. Could the known carotid reflex diminution in adreno-sympathin discharge, *per se* or through some more subtle change in blood chemistry, influence either directly or through local vascularization the metabolism of the central respiratory mechanism to account for the

delayed effects? Or could a gradual reëstablishment of equilibrium between respiratory motor activity and respiratory-muscle proprioceptive influence be involved? Or, most simply, is this reflex characterized by an extraordinarily gradual central recruitment of inhibition, suggesting a very complex or diffuse central nervous pattern? These and other factors may operate together.

Whatever it is fundamentally, it probably operates in nature as a part of the reflex, when arterial hence endosinual pressure is not changing so rapidly or extensively that it could not develop its influence. For this reason we believe that the stepwise procedure (curves G and H) permits a more real representation of natural carotid sinus respiratory depression than the rectangular procedure.

Discussion and conclusions. First, *there is a real carotid sinus pressoreceptive influence on respiratory control,* rather than merely an apparent one arising from altered blood-flow through the carotid gland as suggested by the work of Euler and Liljestrand (1936) and Stella (1936), for in the present experiments the carotid gland was embolized.

Second, it is apparent that *this influence is consistently one of inhibition.* In these experiments pressoreceptor discharge exclusively was altered, by means of the "adequate" stimulus. The capricious excitatory influences reported to follow various mechanical or electrical irritations of the carotid sinus and gland region and mixed sinus nerve need not confuse us.

Third, we believe that *it is as constant and definite an influence as the carotid sinus cardio-vascular reflexes.* Schmidt's (1932) statement that "the intensity of the respiratory response to alterations in endosinual pressure bore no constant relation to that of the circulatory" was based on experiments in many of which the aortic nerves were apparently intact and in all of which arterial pressure and pulmonary ventilation were uncontrolled. In fact, in similar experiments, with aortic innervation intact, we frequently saw respiration relate itself inversely with aortic pressure rather than intracarotid pressure, with instances of striking augmentation of respiration when a rise in carotid pressure elicited a strong arterial hypotension (Winder, 1937a; see also Schmidt, 1932). We have observed respiration to relate itself inversely with rhythmic *oscillations* in arterial pressure following a change in intracarotid pressure, when the aortic nerves were intact.

Fourth, *when other important factors controlling breathing are kept constant, there is a quantitative reflex relationship between the absolute endosinual pressure-stimulus and breathing activity,* as much so as there is between absolute endosinual pressure and heart-rate or vasomotor activity. Apparently Schmidt's (1932) finding that "the respiratory response was essentially a response to change in endosinual pressure, not to any absolute pressure, while the circulatory response was more nearly proportional to

the actual pressure level," represents the influence of secondarily changing factors more powerful in respiratory regulation than in circulatory, which in the uncontrolled animal are free to operate.

Fifth, progressively increased or constant stimulation for long periods indicates that the *carotid pressoreceptive influence on breathing is capable of persisting indefinitely without apparent escape.* Consequently we feel that the commonly observed escape in uncontrolled animals is not a real characteristic of the reflex as emphasized by Schmidt (1932).

Sixth, visualizing a curve relating endosinual pressures and the point for point slopes of the activity curves A to H, which amounts to a first differential or activity coefficient curve, it becomes apparent that *the activity coefficient of the reflex is greatest in the neighborhood of the animal's normal arterial pressure,* which signifies that *physiological* fluctuations in arterial pressure are most powerful in influencing respiration through the carotid pressoreceptive mechanism.

Such is *apparently* not the case in experiments where carotid gland circulation is left patent. In preliminary experiments without embolization of the gland, there were remarkable changes in breathing movements when intracarotid pressure was changed only a few millimeters from or back to zero. These effects were especially pronounced when complete circulatory stasis was insured at zero by use of non-pulsatile pressure. It is precisely what Schmidt (1932) observed in experiments on uncontrolled animals without gland embolization. Since it is eliminated by embolization it now seems almost necessary to believe, as Samaan and Stella (1935) have already suggested, that this result represents at least in major part a carotid gland influence. To assume that it is a function of pressoreceptors in the gland would imply that their thresholds and maximal excitations are almost all attained within a few millimeters of mercury pressure over zero, which is highly improbable; and that their importance approaches that of the sinus, which is not supported by anatomical, physiological or electrical studies. It seems necessary to invoke the recognized chemoreceptor activity of the gland, and this conforms with the observation of Bogue and Stella (1935) and Samaan and Stella (1935) that rather complete circulatory stasis causes a discharge to develop similar to that provoked by asphyxia, CO_2, anoxemia, nicotine or cyanide. It is to be noted that a discharge builds up, instead of disappearing as would probably be the case for pressoreceptors. Apparently more or less complete cessation and reestablishment of circulation in the carotid gland leads to asphyxial excitation and removal of that excitation, respectively. This implies an active metabolism of the large epithelioid cells as suggested by Castro (1926, 1927–1928) on anatomical bases, and brings us to Gesell's (1925, 1929) concept of respiratory control, acting in a peripheral chemoreceptor where changes accompanying alterations in intracellular acidity may lie at the basis of excitation of end-organs (Winder, 1937b; Bernthal, 1938).

The effect on breathing of the stretch-reflex itself, trimmed of foreign influences, thus appears to resemble in considerable detail the corresponding cardio-vascular reflexes. The apparent lack of resemblance in uncontrolled experiments cannot be taken as evidence that the respiratory influence is subserved by a group of end-organs distinct from those for the circulatory influences (cf. Schmidt, 1932). That question remains unsolved.

The *type* of experiment reported here seems adequate to study the *form* of relation between endosinual pressure and its reflex influence on respiration, and the threshold, turning-point and maximal pressures. The quantitative influence of both sinuses, or of both sinuses together with both aortic nerves, responding to a common natural stimulus, arterial pressure, is yet to be investigated, as are modifications of the reflex acting on various central nervous and chemical backgrounds. Until then, the larger significance of the carotid pressoreceptive respiratory reflex in the economy of the organism will remain a matter of speculation.

SUMMARY

A survey of the literature and preliminary experiments led to the belief that a carotid sinus presso-respiratory reflex had not been adequately demonstrated and studied. Uncontrolled qualitative and quantitative changes in carotid gland circulation (fig. 1), uncontrolled arterial pressure and ventilation of systemic blood (fig. 2) were regarded as serious complications in the best of previous experiments.

In the present work, the carotid gland of the anesthetized dog was embolized; the isolated carotid segment perfused and aortic pressure controlled with circulating aortic blood from the animal (fig. 3); pulmonary ventilation controlled during open pneumothorax; vago-sympathetic-aortic nerves severed to eliminate secondary respiratory reflexes and changes in cerebral circulation via that trunk; and respiratory movements recorded plethysmographically.

A stretch-reflex was demonstrated to exist, independent of changes in carotid gland circulation (fig. 6). Whether pulsatile perfusion pressures or blind-end static pressures were used, curves relating endosinual pressure and its influence on minute volume of breathing movements (figs. 4 and 5) were uniformly sigmoid and consistently indicated inhibition, with threshold pressure-stimulus at 15 to 45 mm. Hg, average 20 to 24; maximal pressure-stimulus at 205 to >270 mm. Hg, median 245±; and slope or activity coefficient increasing from zero at the two ends to a maximal at 95 to 150 mm. Hg, average 122 to 125 (normal arterial pressure). The curve-form and three indicated critical points conform (with the possible exception of the threshold pressure-stimulus) with those for the cardiac and vascular carotid sinus pressoreceptor reflexes.

The reflex was capable of persisting indefinitely. In fact, there was a

very gradual, delayed, *added* action (fig. 6), the fundamental nature of which may be manifold. Progressive, stepwise pressure increases (fig. 5), such that this delayed action was given opportunity to develop, yielded about 70 per cent maximal depression, whereas individual rectangular pressure changes from and back to zero (fig. 4) indicated only about 50 per cent *apparent* maximal depression (by one sinus).

A lack of obvious fundamental difference between the sinus pressoreceptive influences on respiration and on the heart and vessels, leaves a lack of evidence for a distinct set of receptors for the respiratory reflex.

A comparison of these results with those of preliminary experiments without carotid gland embolization indicates that the gland is capable of an intense stimulation of respiration due to self-excitation by virtue of its own metabolism, during circulatory stasis.

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A COMPARATIVE STUDY OF THE GROSS AND MICROSCOPIC EFFECTS OF FOLLICLE STIMULATING HORMONE AND ANTERIOR PITUITARY SEX HORMONE ON THE RAT TESTIS

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In 1929 Fluhman reported the finding of a substance in the blood of ovariectomized women which, when injected into the immature female mouse, led to the stimulation of the Graafian follicle. Zondek (1930) soon demonstrated this substance in the urine of ovariectomized women and in women past the menopause. This finding was confirmed by others (Riley, Brickner and Kurzrok, 1933) including Leonard and Smith, who called this substance the follicle stimulating hormone or the follicle stimulating principle of urine (F.S.U.).

While the effects of gonadotropic hormones such as F.S.U. and anterior pituitary sex hormone have been studied on the hypophysectomized rat (Smith and Engle, 1934) comparatively little has been written concerning the effect of these hormones upon the gonads of the normal rat. This paper reports such a study in which both gross and microscopic observations were made.

Eighty (80) albino rats (Mus norvegicus var. albus) of Wistar Institute strain from 30 to 108 days old were used. They were divided into two groups and treated with 1, follicle stimulating hormone from menopausal urine, or 2, anterior pituitary sex hormone. Of each sub-group, 20 were used as experimental animals and 20 as litter mate controls. The groups were further subdivided into those younger than 45 days (the age at which normal testicular descent was considered to occur, Donaldson, 1924) and those older than 45 days.

All animals were kept under similar conditions (Rubinstein, 1932). The test animals receiving *follicle-stimulating* hormone had daily (except Sunday) intraperitoneal injections of 10 rat units of the hormone for 10 days (total of 100 rat units). Those animals receiving the anterior pituitary sex hormone received similar injections of 10 rat units of a weakly aqueous alcoholic extract of sheep anterior pituitaries for 4 days, then 20 rat units daily for 6 days (total of 160 rat units). The control animals received no injections.

The follicle stimulating hormone represented the water soluble fraction of menopausal urine. This had been assayed and standardized for its follicle stimulating effect upon hypophysectomized rats so that each cubic centimeter equaled 25 rat units. The anterior pituitary sex hormone was biologically assayed and standardized for its size and weight-increasing effect of the uterine horns, ovaries, and maturation of Graafian follicles upon the normal immature albino rat. Each cubic centimeter contained 100 rat units. After the ten injections the animals were sacrificed and the body and testicular weights were recorded, averaged and the probable errors calculated for the means. The results obtained for the test animals of each group were compared with the respective controls and differences

TABLE 1

Comparison of testicular and body weights in animals younger than 45 days treated with follicle stimulating hormone and controls

ANIMAL	TESTICULAR WEIGHT	DIFFERENCE	CRITICAL RATIO	BODY WEIGHT	DIFFERENCE	CRITICAL RATIO
	gm.			*gm.*		
Test animals	0.6980±0.0319	0.3381±0.0447	7.6	45.2±2.3	6.4±3.7	1.73
Controls	0.3599±0.0314			51.6±2.9		

TABLE 2

Comparison of testicular and body weights in animals older than 45 days treated with follicle stimulating hormone and controls

ANIMAL	TESTICULAR WEIGHT	DIFFERENCE	CRITICAL RATIO	BODY WEIGHT	DIFFERENCE	CRITICAL RATIO
	gm.			*gm.*		
Test animals	2.8027±0.631	0.4055±0.0406	9.99	176.7±10.4	8.8±12.2	0.72
Controls	2.3972±0.0260			167.9± 7.0		

noted were computed for critical ratios (deviation ± probable errors). All findings were then tabulated as shown in tables 1, 2, 3 and 4.

From table 1 which considers the younger animals treated with F.S.U., it may be seen that the difference in body weight of 6.4 ±3.7 gram in favor of the control is probably insignificant since the critical ratio is only 1.73. The mean testicular weight for the test animals when compared with the corresponding weight of the control shows a difference of 0.3381 ±0.0447 gram, which as judged by the critical ratio of 7.56 is probably significant.

From table 2 which contains results obtained in animals over 45 days of age treated with F.S.U. and their controls it may be noted that the difference in final body weight of 8.8 ±12.2 grams (giving a critical ratio of 0.72) is insignificant. The results obtained for testicular weight, however,

disclose a difference of 0.4055 ±.0406 gram in favor of the test animals (critical ratio of 9.99) which is, therefore, probably significant.

Table 3 reveals the results obtained in animals younger than 45 days treated with anterior pituitary sex hormone and their controls. The difference in mean testicular weight of 0.0158 ±0.0346 gram (critical ratio of 0.4560) is not beyond the probability of random sampling. Body weights for this group likewise show an insignificant difference.

In the group of animals older than 45 days as shown by table 4, significant testicular enlargement was observed since the difference in favor of the test animals was 0.4300 ±0.0510 (critical ratio of 8.4). Comparison of mean body weights shows no significant difference.

TABLE 3

Comparison of testicular and body weights of animals younger than 45 days treated with aqueous extract of anterior lobe of the sheep pituitary with their controls

ANIMAL	TESTICULAR WEIGHT	DIFFERENCE	CRITICAL RATIO	BODY WEIGHT	DIFFERENCE	CRITICAL RATIO
	gm.			*gm.*		
Test animals	0.3441±0.0147	0.0158±0.0346	0.456	44.4±3.5	7.2±4.5	1.6
Controls	0.3599±0.0314			51.6±2.8		

TABLE 4

Comparison of testicular and body weights in animals older than 45 days treated with aqueous extract of anterior lobe of the sheep pituitary with their controls

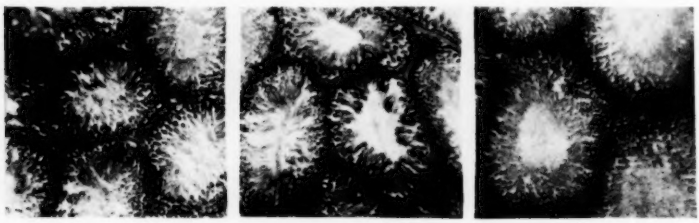
ANIMAL	TESTICULAR WEIGHT	DIFFERENCE	CRITICAL RATIO	TESTICULAR WEIGHT	DIFFERENCE	CRITICAL RATIO
	gm.			*gm.*		
Test animals	2.8272±0.0436	0.4300±0.0510	8.4	167.4±7.8	0.5±10.5	0.04
Controls	2.3972±0.0260			167.9±7.0		

In addition to the testicular weight stimulating effect of these hormones it was noted that testicular descent occurred in the 30 day old test animals of both groups after the third injection. The testes of the control animals remained intra-abdominal until 40 days of age.

The testes of all animals were fixed in formalin, embedded in parlodian, sectioned and stained by hematoxylin-eosin. Microscopic examination of the stained specimens disclosed a marked interstitial tissue increase as well as tubular-cell proliferation in those animals younger than 45 days treated with the follicle stimulating hormone (fig. 2). The control animals of this group showed no increase in the interstitial tissue and little mitotic activity. Animals older than 45 days treated with F.S.U. showed a lesser response in interstitial tissue (fig. 1). The germinal epithelium, however, displayed a marked increase in spermatozoa and an increased

diameter of the tubules. Control animals revealed normal amounts of interstitial tissue and spermatogenesis which was in keeping with the normal.

The testis of the younger group of animals subjected to the anterior pituitary sex hormone (fig. 2) revealed interstitial tissue increase with



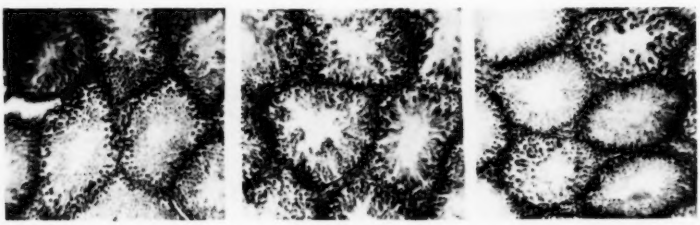
A B C

Fig. 1. Testes of mature rats (photographed at 100 ×). Spermatogenesis is complete in all.

A. Normal.

B. Anterior pituitary sex-hormone treated animal—showing moderate increase in interstitial tissue and complete spermatogenesis.

C. Follicle stimulating hormone treated animal—showing moderate increase in interstitial tissue but markedly increased tubular diameter. Spermatozoa appear to be increased.



A B C

Fig. 2. Testes of immature rats (photographed at 100 ×). Spermatogenesis is incomplete in all.

A. Normal.

B. Anterior-pituitary sex-hormone treated animal—showing moderate increase in interstitial tissue and mild increase of mitotic forms in germinal epithelium.

C. Follicle stimulating hormone treated animal—showing marked increase in interstitial tissue and marked increase of mitotic forms in germinal epithelium.

slight increase of tubular proliferation as gauged by mitotic activity. The response was not as great in this group as in that (group 1) treated with F.S.U. The findings in the older test animals were essentially the same, i.e. slight increase of interstitial tissue and spermatogenic activity.

Discussion. From these experiments it may be noted that both hormones used lead to premature descent of the testis in immature rats. In

this respect, they both resemble the water soluble (pituitary-like) fraction of pregnancy urine (Rubinstein, 1934). Both immature and mature animals when treated with follicle stimulating hormone show significant testicular enlargement. Treatment with the anterior pituitary extract, however, led to significant testicular enlargement only in the older group of animals.

This testicular enlargement results from two factors: 1, increased interstitial tissue; 2, tubular cell proliferation. The increased interstitial tissue and tubular cell proliferation produced by F.S.U. have also been noted in experiments in the hypophysectomized rat (Smith, Engle and Tyndale, 1934). The increase in cell proliferation is not to be confused with a more rapid maturation of germ cells since fully mature spermatozoa were not found in any of the immature animals.

Since the sex hormone used is obtained from the anterior lobe of the pituitary and since F.S.U. is believed by some to be an anterior lobe secretion, it is interesting to note that the products used did not stimulate general body growth. That this lack of growth was not due to a toxic factor in the hormones used may be gathered from the fact that toxic materials not only fail to stimulate growth, but actually inhibit growth. From tables 1 to 4 it may be seen that all sets of animals grew in parallel fashion.

It will be noted that normal instead of hypophysectomized animals were used in these studies. Those who advocate only the use of hypophysectomized animals for such studies as these may feel that this practice insures the subsequent administration of a specific hormone into an animal which secretes none of that hormone for itself. In this way initial deficiencies induced by hypophysectomy are supposedly repaired by a given dosage of material injected.

It is well to realize, however, that since the pituitary gland is in a practical sense a multi-hormonal secreting body pituitary ablation does not only exclude a particular hormone but rather results in the deficiency of all of its hormones. It is obvious, therefore, that a single hormone injected into these defective animals will not be completely substitutive.

This idea is supported by the fact that failure of continued growth results in the hypophysectomized rats treated with a purified growth hormone (Collip, 1934) unless this hormone is augmented by some other substance (Evans, Pencharz and Simpson, 1935).

Hence even the use of hypophysectomized animals has its limitations. It appears, therefore, that in experiments of this nature any data which have been carefully gathered, sufficiently studied and critically analyzed have their usefulness toward final elucidation.

CONCLUSIONS

1. The follicle stimulating hormone produces testicular descent in immature male rats.

2. Anterior-pituitary sex hormone also produces testicular descent in immature rats.

3. Follicle stimulating hormone produces early testicular enlargement in both normal immature and mature animals, while anterior pituitary sex hormone produces testicular enlargement only of the normal mature animal.

4. Follicle stimulating hormone produces marked interstitial tissue increase and marked proliferative activity of germinal epithelium in the gonads of normal immature and mature rats; while germinal proliferation is increased, maturity is not hastened

5. Anterior-pituitary sex hormone produces the same effect as F.S.U. but to a lesser degree.

6. Neither hormone produces general body growth.

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THE EFFECT OF CERTAIN FOODS ON BILE VOLUME OUTPUT RECORDED IN THE DOG BY A QUANTITATIVE METHOD[1]

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The literature is discordant in regard to the effect of various foods on the volume output of bile (1). In fact, doubt has been expressed from the results of studies on human biliary fistula patients as to whether the ingestion of a meal influences bile output (2).

A study of the literature to determine the reason for the variable results revealed several probable causes. Most workers observed fluctuations in volume output, for which they could not account (1, 3). The experimental methods used for the collection of bile as well as the conditions under which a certain food was fed vary widely. In some instances the observation period amounted to only a few hours, in others several days. In some instances bile was fed or allowed by mouth, in others bile was not returned to the intestine at all. Sometimes the gall bladder was left in place and the common duct was transplanted to the abdominal wall; or a gall bladder fistula was made. Frequently the bile passages and liver were or must have been infected, a possibility that must always be considered in the interpretation of results obtained from human patients with a biliary fistula. Even McMaster, Broun and Rous (3) with their method, which avoids hepatitis and cholangitis but does not provide for the return of bile to the intestine, found marked and inexplicable daily variations in volume output.

This investigation was undertaken on the presumption that the formation of bile should be no more variable than the other digestive secretions, and that consistent and reproducible results would be obtained if an adequate method for collecting bile over a period of months was employed and if the experimental conditions were adequately controlled with the animal in a good physiological state.

METHODS. The method of Rous and McMaster (4) was chosen as the best method that had been devised for collecting total bile. Since it was obviously desirable to return bile to the intestine except when the effect

[1] This investigation was facilitated by a grant from Dr. S. W. McArthur.

of withholding bile was to be determined, a tube was passed through the sphincter of Oddi into the intestine for about eight inches. It was found that after two weeks or so the tract formed by the intestinal tube became connected with the tract formed by the tube in the common duct and infection ensued. This was obviated by inserting the intestinal tube through a small opening in the duodenal wall about three inches distal to the sphincter of Oddi. But, during a fifteen-month period of experimentation under controlled conditions, the volume output of bile varied widely and we were unable to obtain consistent and reproducible results. After searching intensively for the cause of the variation, it appeared that it was due to temporary or intermittent obstruction of the tube with biliary sediment and viscous bile. It was then decided to avoid this apparent difficulty by applying continuous suction throughout the day and night. Following the application of this principle, the uncontrollable irregularities in volume output disappeared and infection did not occur provided certain operative details regarding the placement of the tubes were practiced and provided the animals received attention day and night.

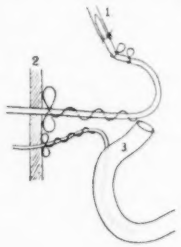

Fig. 1. This figure shows the points at which the pieces of silver wire surround the tube in the common bile duct, and in the duodenum. *1*, the common bile duct; *2*, the abdominal wall; *3* the duodenum.

Operative procedure. Female dogs, weighing between 9 and 13 kilos are used. Under morphine-ether anesthesia, the gall bladder is removed. The common duct is cannulated with a white rubber tube 27 in. long, having a $\frac{3}{32}$ in. bore and a wall $\frac{1}{16}$ in. thick. This tube is serviced with no. 16 silver wire as shown in figure 1, circular grooves being ground where the two figure of eight pieces near the duct are fastened about the tube. Omentum covers these figure-of-eight pieces and prevents the tube from leaving the duct. The longer coil of silver wire serves as a device to prevent the tube from being pulled out and from working to and fro. The distal end of the common duct is tied and buried in the duodenal wall. The tube for the return of bile to the intestine is also serviced with a coil of wire (fig. 1) and is fastened in the duodenum about three inches below the ampulla of Vater with a purse-string suture. These tubes are looped in the abdomen so that they do not touch the liver and are separated by omentum to prevent the tract formed about one tube from becoming continuous with the other.

Each animal is fitted with a harness which is fastened to the side of the cage so

that considerable freedom in the cage is permitted with the exception that turning in a circle is prevented. The tubes, one leading bile from the liver and the other to the intestine, are placed in a "coilette". The "coilette" is serviced with a weighted pulley, which takes up and allows slack with each movement of the animal. The tube passing to the intestine is connected with a burette. The tube draining bile from the liver is connected with a stoppered, graduated (100 cc.) cylinder. A water bottle so arranged as to create suction is also connected with the cylinder. (It was shown in acute experiments that such suction does not increase bile formation by the liver.) With this arrangement the bile can be seen to drop into the cylinder and readings made without disturbing the animal or its dressing. The cylinder may be disconnected from its stopper and the bile poured into the burette to run by gravity into the intestine at a timed rate. The animals were dressed once daily and received attention throughout the day and night.

The amount of bile secreted during a 6-hour period was noted. Hourly readings were made usually from 6:30 a.m. to 12:30 a.m. and only occasionally from 12:30 to 6:30 a.m. Except during the various experimental periods, the animals were fed a weighed portion of a mixed diet consisting of Pard (a commercial dog food; 10.5 per cent protein, 10 per cent carbohydrate, and 2.5 per cent fat) and skimmed milk (100 cc.) every six hours. Sixty cubic centimeters of hepatic bile were returned every six hours immediately after the meal at a rate of 3 cc. per minute, the tube being washed with 100 cc. of water given at the same rate; otherwise water was allowed ad libitum. The amount of bile returned was chosen because it (60 cc.) was the amount usually secreted during a 6-hour period and because one could not expect to obtain quantitative outputs unless a fixed amount of bile was returned. After an experimental variation of this regime, the animals were always returned to the regime for a period before another variation was introduced. No animal was deprived of bile for a period longer than one week.

Only those data obtained from healthy animals, which showed no bile by chemical test in their urine, which ate normally and voluntarily all that was offered them, and whose output on the mixed diet returned to the control level after an experimental variation in diet, will be presented. When bile was not returned to the animal for a period, the stools were examined for bile. None was found in the animals whose data are reported.

RESULTS. *The constancy and reproducibility of the same bile volume output under similar experimental conditions.* An inspection of table 1 will demonstrate that the bile volume output under the three conditions listed (no food and no bile, no food with the return of bile, and a mixed diet with a return of bile) did not vary more than ± 4 per cent for the 24-hour or 12-hour (day and night) periods. This was true of all the animals used in this study, the typical data on dogs 2, 3 and 4 only being submitted because they were observed the longest (4 to 6 months).

TABLE 1

Showing the reproducibility of bile volume output in the same dog under similar conditions

The different periods are separated by five days or more than one week

DOG NO.	SIX HOUR PERIODS	BILE OUTPUT IN CUBIC CENTIMETERS					AVG. DAY AND NIGHT VOL.
		1st expt. period	2nd expt. period	3rd expt. period	4th expt. period	5th expt. period	
	No food and no bile						
II 9.5 kilos	a.m. to p.m.	25	22	22	20	23	
	p.m. to p.m.	22	25	25	22	21	D. 22.7
	p.m. to a.m.	21	23	20	21	26	
	a.m. to a.m.	24	25	23	24	24	N. 23.1
	Totals	92	95	90	87	94	
	50 cc. bile—no food			60 cc. bile—no food			
	a.m. to p.m.	52	48	58	61	60	
	p.m. to p.m.	54	47	58	62	57	D. 50.2, 59.3
	p.m. to a.m.	51	50	62	62	62	
	a.m. to a.m.	54	52	61	56	68	N. 51.9, 61.8
	Totals	211	197	239	241	247	
	125 grams of Pard—100 cc. milk—50 cc. bile						
	a.m. to p.m.	65	66	62	66	62	
	p.m. to p.m.	61	64	60	65	60	D. 63.4
	p.m. to a.m.	64	65	65	61	65	
	a.m. to a.m.	66	63	63	64	63	N. 63.9
	Totals	256	258	250	256	253	
III 11 kilos	No food and no bile						
	a.m. to p.m.	24	23	25	23	26	
	p.m. to p.m.	25	26	26	27	24	D. 24.9
	p.m. to a.m.	23	24	27	25	27	
	a.m. to a.m.	25	27	24	26	23	N. 25.1
	Totals	97	100	102	101	100	
	No food—60 cc. bile						
	a.m. to p.m.	63	66	61	64	59	
	p.m. to p.m.	64	63	60	61	59	D. 62.0
	p.m. to a.m.	61	60	61	62	57	
	a.m. to a.m.	62	64	59	62	56	N. 60.4
	Totals	250	253	241	249	231	

TABLE 1—*Concluded*

DOG NO.	SIX HOUR PERIODS	BILE OUTPUT IN CUBIC CENTIMETERS					AVG. DAY AND NIGHT VOL.
		1st expt. period	2nd expt. period	3rd expt. period	4th expt. period	5th expt. period	
	170 grams Pard—100 cc. milk—60 cc. bile						
III	a.m. to p.m.	71	71	72	69	70	
11	p.m. to p.m.	71	71	72	70	74	D. 70.1
kilos	p.m. to a.m.	73	73	68	69	70	
	a.m. to a.m.	69	70	70	72	74	N. 70.8
	Totals.....	284	285	282	280	288	
	No food and no bile						
	a.m. to p.m.	24	20	22	21	18	
	p.m. to p.m.	23	20	22	22	20	D. 21.2
	p.m. to a.m.	22	21	20	20	21	
	a.m. to a.m.	21	19	18	24	22	N. 20.8
	Totals.....	91	84	82	87	81	
	No food—60 cc. bile						
IV	a.m. to p.m.	59.5	60	60	60	60	
13	p.m. to p.m.	64	59	64	64	64	D. 62.4
kilos	p.m. to a.m.	64	61	53	68	62	
	a.m. to a.m.	58	63	58	58	60	N. 60.5
	Totals.....	245.5	243	235	250	246	
	170 grams Pard, 100 cc. milk, 60 cc. bile						
	a.m. to p.m.	76	77	78	80	78	
	p.m. to p.m.	83	80	84	82	79	D. 79.7
	p.m. to a.m.	84	82	83	80	83	
	a.m. to a.m.	78	79	81	79	80	N. 80.9
	Totals.....	321	318	326	321	320	

To further indicate the reproducibility of the results obtained with the method, the average 24-hour output of dog 1 on the mixed diet (vide ut supra) with the return of bile for the various experimental periods was 243 cc. ±4 per cent; of dog 2, 256 cc. ±3.3 per cent; of dog 3, 283 cc. ±2 per cent; of dog 4, 322 cc. ±2 per cent; of dog 5, 263 cc. ±2.5 per cent; of dog 6, 258 cc. ±2 per cent; of dog 7, 256 cc. ±2 per cent. The averages are based on the daily outputs of from four to eleven different experimental periods.

Such consistently reproducible results have apparently never before been attained by any other method. It should be added that with any

change in diet or in the amount of bile returned, from 12 to 48 hours, occasionally longer, lapse before stabilization in the 6-hour output occurs. The averages given above include only those outputs of the days after stabilization occurred.

We attribute the constancy and reproducibility of the results obtained to the continuous day and night suction applied to the drainage tube inserted into the common duct and to the controlled conditions under which the experiments were conducted. The worth of the data obtained in this study, when compared with that of the older literature, lies chiefly in its quantitative character, the different experimental procedures or "test-meals" being repeated with the same results on the same animal a number of times, over a period of several months. *The data obtained show that the liver under controlled conditions is as constant in its volume output of secretion as any of the external secretory glands of the body, possibly more so.*

McMaster, Broun and Rous (3) observed a bile volume output of from 3.5 to 9.5 cc. per kilo body weight per day. Using their method an output on a mixed diet of from 9 to 20 cc. per kilo per day has been obtained in our laboratory (7). In the Rous method no bile is returned to the intestine. The output of our animals on receiving no food and no bile for a period of from 3 to 5 days, ranged from 6 to 10 cc. per kilo per day. On receiving a mixed diet four times daily without bile, the output ranged from 13 to 18 cc. per kilo. On the same diet with the return of bile to the intestine, the output ranged from 24 to 27 cc. per kilo in the different animals (seven). We attribute the larger outputs observed with our method to the return of bile and to the prevention of temporary partial obstruction. The larger outputs could not reasonably be attributed to cholangitis because our animals did not show bilirubinuria and because under the same experimental conditions the output was constant over a period of from three to six months.

A discrepancy exists in the literature (3, 5) in regard to the effect of hot weather on bile volume output. Our observations indicate that such a disagreement is due to the effect of the weather on the appetite of the animal. The reproducibility of the results was not altered during very warm weather, if the animals ate their food with relish. When the animals did not eat all the food offered, the outputs were not considered to be normal and are not included in the above averages or in any data to be presented later. Further, the quantity of the mixed diet required for the maintenance of each animal must be determined at the outset, because if too much is fed, after a few days the animal will refuse a portion of some of the meals or not consume all of the food at one time, and meaningless alterations in output occur. In regard to the effect of water on bile output, we could never show, when an animal was stabilized to a certain regime, that water influenced the output of bile. When the animal was not

thirsty, we introduced water (100–200 cc.) into the intestine; it had no effect on bile output. The effect of water in the presence of thirst was not determined. Our observations concerning water confirm those of Barbera (6).

Bile secretion in response to a mixed meal with and without the return of bile. In table 2 are shown the volumes of bile collected hourly from three dogs after the feeding of a mixed diet. The data, which are typical and not averages, show that the maximum output occurs during the second hour in those experiments in which bile was returned. (For

TABLE 2

Showing bile secretion with a mixed diet plus bile, mixed diet without bile, and no food or bile

PERIODS OF TIME	DOG 2 CC. BILE			DOG 3 CC. BILE			DOG 4 CC. BILE		
	Mix. diet, with bile 50 cc.	Mix. diet, no bile	No food, no bile	Mix. diet with bile 50 cc.	Mix. diet, no bile	No food, no bile	Mix. diet with bile 60 cc.	Mix. diet, no bile	No food, no bile
a.m. to p.m.	65	40	25	71*	44	25	76	40	24
p.m. to p.m.	61	44	22	71	43	22	83*	40	23*
p.m. to a.m.	64	42*	21	73	42	21*	84	48	22
a.m. to a.m.	66*	46	24*	69	42*	25	78	47*	22
Totals.....	256	172	92	284	171	93	321	175	91
HOURLY AM'TS.									
1	11*	7*	2*	16*	7*	3*	18*	8*	4*
2	24	7	3	24	8	5	29	8	5
3	9	7	5	12	6.5	3	11	8	3
4	8	9	4	9	6.5	3.5	9	6	4
5	7	4	5	5	5	3.5	8	8	4
6	7	8	5	5	9	3	8	9	3
Totals.....	66	42	24	71	42	21	83	47	23

* The hourly reading for this six-hour period.

Body weight of dog 2, 9.5 kilos; dog 3, 11.0 kilos; dog 4, 13.0 kilos.

Dog 2 received 125 grams Pard and 100 cc. of skimmed milk every six hours.

Dogs 3 and 4 received 170 grams Pard and 100 cc. of skimmed milk every six hours.

averaged results see table 9.) If an animal was fasted for three or four days and then was fed the mixed diet every six hours without the return of bile, the hourly rate of secretion increased the first hour after the first feeding; and then continued at the higher feeding level during subsequent 6-hour feedings. Thus, a mixed meal without the return of bile unquestionably stimulates bile output (table 2), but the effect of the meal is prolonged over a period of five or more hours, so that the ingestion of a second meal, no bile being returned, has little or no additive effect. (For

averages see table 9.) That is, a six-hour feeding schedule without bile entering the intestine apparently maintains the rate of intestinal digestion and of bile output at a level higher than the fasting, no-bile level; so, a very definite postcibal curve of secretion is not observed.

We believe that this is the reason why some (2) have failed to observe a definite postcibal increase in bile volume output in patients with a biliary fistula when the patient is fed every six hours without the return of bile. It is evident that the very definite second-hour postcibal increase when bile is returned is due to the absorption or the effect of the bile (see tables 2 and 9).

However, when a larger meal is fed three times a day, or every eight hours, a definite postcibal secretory curve results when no bile is returned to the intestine with the meal. In figure 2 are shown the typical results of feeding a mixed meal of somewhat different composition and of larger

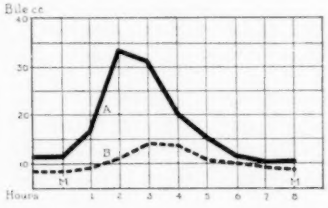

Fig. 2. Showing the rate of secretion of bile on feeding a mixed meal. *M*, 250 grams of a mixed meal (caloric value, 345 calories), consisting of 12.0 per cent protein, chiefly meat, 6.0 per cent of fat, 8.75 per cent of carbohydrate, to a previously fasted (7 days) dog; curve *A*, with the return of bile (120 cc.; 60 cc. the first hour, 45 cc. the second, and 15 cc. the third), and curve *B*, without the return of bile.

quantity than the meal fed on the 6-hour schedule. The animals in this experiment were fed every eight hours or three times daily, after they had received no food and no bile for from 3 to 7 days. The curves shown were those obtained as a result of the first feeding after the fasting period. In these experiments the effect of the meal fed was evident for six or seven hours.

The output in response to a single large meal compared with that of a small meal every six hours. To make such a comparison 75 grams of the mixed diet (Pard) were fed to dog 3 with the return of 60 cc. of bile every six hours for a period; then 300 grams of the mixed diet were fed at one feeding with the return of 60 cc. bile, the bile being returned every six hours. The typical results are represented by figure 3. Dog 2 was fed 100 grams of the mixed diet every six hours with the return of 60 cc. of bile; then a single feeding of 400 grams of the mixed diet was given with the return of 60 cc. of bile every six hours. The results of the 6-hour feedings were, in

sequence: 72, 70, 72, and 74 cc., *a total of 288 cc.* Then the single feeding was fed; the results were, in sequence: 86, 73, 41 and 42 cc., *a total of 242 cc.* In dog 4 the results for the frequent feedings were, in sequence: 74, 72, 71 and 75 cc., *a total of 292 cc.*; for the single feeding: 82, 85, 59 and 50 cc., *a total of 276 cc.* In another experiment on dog 3, 100 grams of the mixed diet were given every six hours with the return of 20 cc. of bile, and then 400 grams of the diet were given with 80 cc. of bile, no bile being given during the succeeding 6-hour periods. The typical results are illustrated by figure 4.

The results show that on keeping the caloric intake of a mixed diet constant for a 24-hour period the quantity of bile secreted on a 6-hour feeding schedule is larger than on a 24-hour single feeding schedule. Also, when one meal is fed during a 24-hour period and bile is returned every

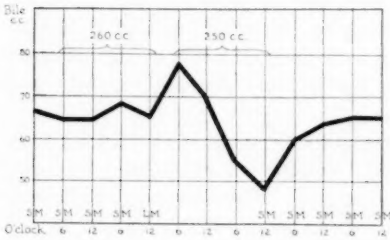

Fig. 3

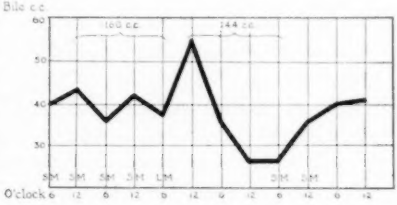

Fig. 4

Fig. 3. Showing the effect of bile volume output of a small mixed meal, *SM*, fed every six hours with the return of bile to the intestine, and the effect of four small meals combined into a large meal, *LM*, with the return of bile to the intestine.

Fig. 4. *SM*, 100 grams of a mixed meal and 20 cc. of bile returned to the intestine. *LM*, 400 grams of a mixed meal and 80 cc. of bile returned to the intestine, and no bile returned at the succeeding 6-hour periods.

six hours, the effect of the meal on bile output is evident for twelve hours. The maximum output occurs during the first six-hours. The point just made is true when the bile is returned every six hours following the single large feeding; but when the bile is not returned, the meal effect does not last much longer than seven or eight hours. These data on the period of time that a meal influences bile output confirm those of Barbera (6), who reported a period of from 10 to 12 hours. Whether a meal is fed with or without the return of bile, the maximum effect of the meal is exerted during the first six hours. This observation confirms most of the reports in the literature with exception of those of Heidenhain and Dastre who reported a maximum output at 3 to 5 hours and another about 12 to 15 hours after a meal (1).

We have never observed the marked diminution or cessation in the secre-

tion of bile immediately after the ingestion of any food as was reported to occur by Okada (9) and Barbera (6), unless the animals were nauseated (salivation and evident discomfort) by eating too much or too hurriedly. Either such must have been true of their dogs or some pressure must have been temporarily exerted on the cystic duct of their gall-bladder fistula animals.

The effect of feeding the mixed diet three times during the day-time on the twenty-four hour output. It is evident from the results in table 1 that when the animal is on a 6-hour feeding schedule no difference between the day and night output exists. Since the ingestion of food and the passage of bile into the intestine stimulate bile output, it is obvious that if an animal is fed three times during the day-time (6:30 a.m., 12:30 p.m. and 6:30 p.m.) and not at night, the night volume should be less. Such data were collected not only to test the foregoing assumption, but also because such a feeding schedule is usually analogous to that of man. For this purpose the mixed diet was used, the 12:30 a.m. feeding and return of bile being omitted; in another experiment the 12:30 a.m. feeding was omitted, but bile was returned.

The results are shown in table 3. It is to be noted that with no food at midnight but with the return of bile, the night-output in five dogs was from 7 to 16 per cent less than the day-output. When no food or bile was given at midnight, the night-output in three dogs was from 20 to 25 per cent less than the day-output. This is similar to the observations made on some human patients with biliary fistula (2). It is to be noted again (compare data on dogs in table 2 with those in tables 3 and 1) that the effect of the 6:30 p.m. meal carries over into the post-midnight period in practically every case.

A disagreement exists in the literature (1) in regard to the bile output during the day and night. The results with our method show that if the animal is on a six-hour feeding schedule, no difference exists. If the animal receives no food and no bile, a difference does not exist. If the animal receives no food but bile every six hours, no difference exists. However, if the animal is fed either one or three meals during the day and no food during the remainder of the 24-hour period, the bile secreted during the night is less (table 3, and figs. 1 and 2). This is the response that should be expected, if a meal has any effect on bile-volume output, and if the liver does not manifest some peculiar diurnal variation as suggested by Forsgren (8).

The effect of the administration of bile alone and of a mixed diet alone is not strictly additive. This is shown by the data in table 4, which presents the averages of four or more determinations for each of the four experimental conditions. This was not true of a meal of raw ground meat, however (vide ut infra).

TABLE 3

Showing the day and night volume output of bile (averages) on the ingestion of food (mixed diet) three times daily at 6:30 a.m., 12:30 and 6:30 p.m. with and without the return of bile at 12:30 a.m.

DOG. NO.	BILE OUTPUT IN CC.						PER CENT DECREASE OF NIGHT OUTPUT
	6:30 a.m. to 12:30 p.m. A	12:30 p.m. to 6:30 p.m. B	6:30 p.m. to 12:30 a.m. C	12:30 a.m. to 6:30 a.m. No food D	Day output A + B	Night output C + D	
With return of bile at 12:30 a.m.							
1	63	62	60	45	125	105	16
2	64	60	63	49	124	112	9
3	72	69	73	57	141	130	8
4	77	80	81	64	157	145	7
10	65	66	64	47	131	111	15
Without the return of bile at 12:30 a.m.							
1	57	59	59	28	116	87	25
3	70	74	70	44	144	114	20
4	84	83	81	49	167	130	22

TABLE 4

Showing that the stimulating effect of a mixed diet alone and bile alone on bile formation are not strictly additive

DOG. NO.	BILE OUTPUT PER DAY							DIET EFFECT PLUS BILE EFFECT A + B*
	No food, no bile, cc.	No food, 60 cc. bile		Mixed diet,† no bile		Mixed diet, 60 cc. bile		
		Cubic centimeters	Increase over basal, cc. A	Cubic centimeters	Increase over basal, cc. B	Cubic centimeters	Increase over basal, cc. C	
2	92	242	150	172	80	285	193	230
3	100	245	145	171	71	284	184	216
4	85	246	161	175	90	321	236	251

* The values in column C might be expected to equal the values of column A + B.

† One hundred and twenty-five grams Pard and 100 cc. of skimmed milk.

TABLE 5

The effect of meat with and without the return of bile on bile volume output

Dog 2

PERIOD OF TIME	BILE OUTPUT IN CC.				
	No food, no bile	125 gm. beef, no bile	No food, 50 cc. bile	125 gm. beef, 50 cc. bile	225 gm. beef, 50 cc. bile
a.m. to p.m.	22	32	52	62	78
a.m. to p.m.	25	38	54	70	78.5
p.m. to a.m.	23	40	51	74	74
a.m. to a.m.	25	39	54	71	76
Totals	95	149	211	277	306

It is to be noted (table 4) that 60 cc. of the dog's hepatic bile is about twice as potent a stimulus for bile formation as the mixed ration used.

The effect of meat on bile volume output. To study the effect of meat on bile volume output, "inside beef ham" was chosen because of its relatively high protein content; an adequate supply of uniform grade was available for the tests. The ground beef (125 grams; protein 22.4 per cent; fat 2.9 per cent; 140 calories) was fed raw with and without the return of bile to the intestine to four animals. The effect of cooking the beef was not determined.

The results on one of the animals are shown in table 5. The other animals responded similarly. The meat without the return of bile stimulated bile output; the maximum output resulted either during the second or third hour after feeding. When bile was returned the maximum output occurred during the second hour. (For averages see table 9.) The effect of the raw meat and of the bile was definitely additive in all dogs. Why this was true of the meat (140 calories) and not for the mixed ration (170 calories) is not clear. (Compare results on dog 2, tables 2 and 5, and the averages in table 9.)

The effect of feeding meat more frequently than every six hours. The effect of feeding the meat more frequently than every six hours was determined on two animals. Typical results are shown in table 6. It is to be noted that when the usual 6-hour ration of meat and bile is divided into two or three-hour portions and fed, the 6 or 18-hour output is not altered. From a practical viewpoint, this observation is of interest. *However, it should be recalled that our animals were devoid of a gall bladder, and it is possible that with a functioning gall bladder in place the result, because of an intermittent discharge of bile, might be different.*

The effect of adding meat to a mixed diet. In two dogs standardized to the mixed diet with the return of bile, 125 grams of beef were added to each 6-hour feeding. This increased the bile output for each 6-hour period by 27 per cent in one and 15 per cent in the other. When the animals were returned to the mixed diet, the stimulating effect of the meat disappeared in six hours.

The effect of olive oil (fat) on the output of bile. The effect of feeding olive oil with and without bile as well as the addition of olive oil to the mixed diet was determined on four animals. It should be mentioned that numerous experiments on this subject had to be performed because the continued administration of olive oil alone frequently led to vomiting which, when it occurred, rendered the results valueless for our purpose. Only the data from tests in which no vomiting or lack of appetite (dogs lapped the oil) for the oil was manifested will be presented. The animals would only tolerate the oil in 20 cc. quantities every six hours. Larger quantities produced vomiting or anorexia. We used olive oil rather than butter,

which is the fat most frequently used in the studies in the literature, because the composition of olive oil varies less than butter.

The data for two of the four dogs are shown in table 7. When olive oil was fed with the return of bile, the bile output per 6-hour period was augmented by from 8 to 20 cc. in the different dogs in different tests. The increase was usually most definite after the first feeding except in dog 3. The maximum output was reached during the second or third 6-hour period after the feeding of the oil was started. In dog 4 the increase was prompt and maintained throughout the feeding of the oil and the effect of the oil continued for six hours after it was no longer fed. In dog 6 the response to the oil was prompt; the increase disappeared promptly after

TABLE 6

Showing the effect of feeding meat with the return of bile more frequently than every six hours for eighteen hours

Bile output in cc.

125 GM. BEEF, 50 CC. BILE, EVERY 6 HOURS	62.5 GM. BEEF, 25 CC. BILE, EVERY 3 HOURS	125 GM. BEEF, 50 CC. BILE, EVERY 6 HOURS	42.3 GM. BEEF, 16.6 CC. BILE, EVERY 2 HOURS
			18
	33.5		25
	38.0		24
74	71.5	71	67
			23
	39		22
76	34		22
	73	73	67
			26
	38		25
72	36		28
	74	69	79
Totals 222 cc.	218.5 cc.	213 cc.	213 cc.

feeding of the oil was stopped. In dog 3 the response was gradual and the output decreased to the fasting level during the continuance of the feeding of the oil. In dog 2 the increase was prompt and continued at the increased level for 18 hours, and then fell to the fasting level, although the animal still received the oil. The averaged hourly response is shown in table 9.

When the oil was fed without the return of bile, the stimulation of bile output was evident, but not so marked as when bile was returned. The stimulating effect of the oil was not maintained well, and fell to the no-food, no-bile level during the third 6-hour period of oil feeding in three of the four dogs. The averaged hourly response is shown in table 9.

The effect of the addition of 60 cc. of olive oil to a mixed diet with the return

of bile on bile volume output. In three of the animals after they had been standardized on a mixed diet with the return of bile, 60 cc. of olive oil were added to one of the six hour feedings. Dog 1 on the mixed diet secreted 62, 63, and 63 cc. of bile per 6-hour period; on the addition of the

TABLE 7

Showing the effect of olive oil with and without the return of bile on the output of bile in two of the four dogs used

DOG 2				DOG 3			
With 60 cc. bile returned	Bile, cc.	No bile returned	Bile, cc.	With 60 cc. bile returned	Bile, cc.	No bile returned	Bile, cc.
No oil	60	No oil	23	No oil	58	No oil	23
No oil	62	No oil	25	No oil	59	No oil	26*
No oil	58	No oil	20	No oil	57*	No oil	24
No oil	58*	No oil	23	No oil	56	No oil	27
20 cc. oil	68†	20 cc. oil	36.5†	20 cc. oil	64	20 cc. oil	34
20 cc. oil	69	20 cc. oil	29.5	20 cc. oil	64.5	20 cc. oil	34†
20 cc. oil	72	20 cc. oil	26.0	20 cc. oil	68†	20 cc. oil	26
20 cc. oil	61.5	20 cc. oil	21.0	20 cc. oil	61	20 cc. oil	29
No oil	63	No oil	18*	20 cc. oil	61	20 cc. oil	26
No oil	62	No oil	17	No oil	61	No oil	24
No oil	62	No oil	16	No oil	59	No oil	27
		No oil	19.5	No oil	58	No oil	23
			Hourly reading				
1	8*	3*		10*		4*	
2	14	3		13		5	
3	14	3.5		12		5	
4	9	2.5		10		3	
5	9	2.5		6		4.5	
6	4	2.5		6		4.5	
	58	18		57		26	
1	12†	7†		18†		6†	
2	21	7		18		5.5	
3	13	6		16.5		6	
4	9	6.5		5.5		4.5	
5	7	5.5		6		6.5	
6	6	4.5		4		5.5	
	68	36.5		68		34	

oil, 63 cc.; then on the diet without oil, 62, 64 cc., etc. Dog 2 on the mixed diet with the return of bile secreted 65, 60, and 65; on the addition of the oil, 65 cc.; then on the diet without oil, 64, 65 cc., etc. Dog 6 on the mixed diet with the return of bile secreted 65, 64, 62, and 62 cc.; on the addition of the oil, 63.5 cc.; then on the diet without oil, 66 cc., etc. A repetition

of the experiment yielded a similar result. Thus, adding olive oil to the mixed diet had no effect on bile volume output.

It is difficult to compare our results on olive oil with the effect of fat on bile output observed by others, because the fat fed was different and it was fed under different conditions. Our results show without doubt that the feeding of olive oil alone to a fasted animal, receiving or not receiving

TABLE 8

Showing the effect of glucose intravenously on bile output with and without the return of bile; also the effect of a NaCl control

Thirty-five grams glucose in 200 cc. 0.5 per cent NaCl given at the beginning of the six hour periods during 30 min.

DOG 2, 9.5 KILOS						DOG 3, 11 KILOS					
60 cc. bile	Bile, cc.	No bile	Bile, cc.	60 cc. bile	Bile, cc.	60 cc. bile	Bile, cc.	No bile	Bile, cc.	60 cc. bile	Bile, cc.
No glucose	58	No glucose	23	No NaCl	58	No glucose	61	No glucose	23	No NaCl	64*
No glucose	62	No glucose	21	No NaCl	57	No glucose	60	No glucose	27*	No NaCl	61
No glucose	62	No glucose	26	No NaCl	62	No glucose	61	No glucose	25	No NaCl	62
No glucose	56*	No glucose	24*	No NaCl	58†	No glucose	59*	No glucose	26	No NaCl	62
Glucose	69	Glucose	22	NaCl	58†	Glucose	84†	Glucose	23†	NaCl	59†
Glucose	80†	Glucose	24	NaCl	62	Glucose	69	Glucose	20	NaCl	63
Glucose	60	Glucose	15†	No NaCl	60	Glucose	52	Glucose	21	NaCl	64
Glucose	57	Glucose	17			No glucose	47	No glucose	19	No NaCl	60
No glucose	57	No glucose	15			No glucose	53	No glucose	18	No NaCl	62
No glucose	62	No glucose	19			No glucose	60	No glucose	21		
No glucose	58	No glucose	20			No glucose	61	No glucose	24		
No glucose	58										
Hourly amounts											
9*		8*		8*		10*		4*		11*	
16		3		14		10		6		14	
13		5		13		24		5		21	
8		1.5		10		3		5		7	
5		3.5		9		5		4		5	
5		3		4		7		3		4	
56		24		58		59		27		64	
13†		1.5†		10†		12†		3†		10†	
17		1.5		18		34		7		17	
22		4		14		25		2		15	
14		2		5		4		4		6	
7		3		3		4		4		5	
7		3		8		5		3		6	
80		15		58		84		23		59	

bile, stimulates bile output. This observation confirms several reports (1). The stimulation of bile output by olive oil does not continue as long as the olive oil is fed. If no bile is returned, the olive oil fails to stimulate after one or two feedings at 6-hour intervals. If bile is returned, the oil stimulates for at least three successive 6-hour feeding periods. The stimulating effect of a single feeding of olive oil (60 cc. 2 expts.) with the

return of bile lasts for from 6 to 8 hours. This is in contrast to the length of the period of stimulation reported by Barbera (6) for 100 grams of butter; he reported the length of the period to be 19 to 20 hours. Also, the butter curve as observed by Barbera reaches a maximum at the 5th and 6th hour. In our experiments the maximum response usually occurred during the second hour. But, we used a smaller quantity of a different fat.

Concerning the effect of the addition of fat to a mixed diet, the literature shows no agreement, some investigators reporting an increase, others

TABLE 9

Showing the hourly secretion of bile after the ingestion of various foods with and without the return of bile to the intestine

MEAL FED	HOUR BEFORE FEEDING	1	2	3	4	5	6	TOTAL	CHANGE DUE TO MEAL
Raw meat and bile; 140 cal.	6.0	15.2	18.8	11.1	8.7	6.7	6.8	67.3	+15.3
Raw meat and no bile; 140 cal.	4.0	5.0	5.9	6.0	5.2	5.5	5.9	33.5	+9.5
Mixed diet (meat cooked) and bile; 170 cal.	6.0	12.4	22.9	9.2	7.8	5.9	5.9	64.1	+12.1
Mixed diet (meat cooked), no bile; 170 cal.	4.2	7.3	7.1	6.8	6.8	5.6	7.1	40.7	+16.7
Olive oil and bile; 180 cal.	5.8	10.0	19.1	12.1	7.3	8.0	6.7	63.2	+11.2
Olive oil, no bile; 180 cal.	4.0	5.0	7.0	4.1	4.3	5.7	4.8	30.9	+6.9
Oral glucose and bile; 140 cal.	6.0	9.5	16.5	10.5	5.0	4	4.0	49.5	−2.5
Oral glucose, no bile; 140 cal.	4.2	3.5	3	3	3	2.5	3	18	−6.0

The data shown (table 9) are averages of the responses of four dogs. When bile was returned, the amount returned amounted to 50 cc. of hepatic bile. The foods were fed, or the experiments were performed, after the animals had become stabilized (3–5 days) on no food and no bile. The average bile output on no bile and no food was 24 cc.; on no food and 50 cc. of bile, 52 cc.

a decrease in bile output. Since we obtained no effect, one might attribute our observations to the method employed; all others have used the gall bladder-fistula method. Our failure to observe an increase in output under quantitative conditions indicates to us that an increase in the caloric value of a diet alone (addition of fat) is not a factor in augmenting bile output. In this connection it should be recalled that adding 140 calories in the form of 125 grams of meat to a mixed diet increased bile output, but adding 180 calories in the form of fat did not. The explanation of this difference might appear to be that 125 grams of meat is a more potent, specific food stimulus than 20 cc. of olive oil. But, in dog 2 (compare data in tables 5 and 7) when oil and meat were fed alone with or without the return of bile, 20 cc. of the oil were as potent as 125 grams of

raw meat. This was not true when all the data on the four dogs studied are averaged; the meat was slightly more potent than the olive oil (table 9). Twenty cubic centimeters of olive oil added to a mixed diet would tend to retard gastric evacuation and this effect of the oil renders a precise interpretation difficult. Since the action of fat on the digestive tract is complex, and subject to individual variation, the reports in the literature that fat added to a diet sometimes stimulates slightly, or may have no effect or may decrease bile output is not surprising.

It may be concluded from our observations that: *a*, the ingestion of olive oil by a fasting animal, with or without the return of bile to the intestine, stimulates bile output in most but not all animals to the same extent as 125 grams of raw ground meat; *b*, the stimulating action of olive oil disappears when it is repeatedly ingested at 6-hour intervals, and the disappearance of its stimulating effect occurs sooner when bile is not returned to the intestine; *c*, when 60 cc. of olive oil are added to a mixed diet, it has no effect, the stimulating effect of the fat when fed alone being masked probably by the action of fat on the motility and secretion of the stomach, which also modifies the effect of the mixed diet on bile output. Thus, olive oil is not a constantly reliable excitant of bile volume output except when fed once or twice after a period of fasting; this follows from our own observations and is suggested by the disagreement in the literature.

The effect of glucose orally on bile volume output. To determine the effect of glucose on bile volume output in fasting, 35 grams of glucose were given orally (voluntary ingestion) in 250 cc. of water with and without bile being returned. Four animals were used. As in the experiments on meat and olive oil the animals were stabilized at a fasting level with or without bile being returned, then the glucose with water was given for three or four consecutive 6-hour periods; and, then the glucose was not given for three or four 6-hour periods.

With bile being returned, dog 2 showed an increase of 6 cc. during the third 6-hour period; dog 3 showed no change; and dogs 4 and 5 showed a definite decrease. The control output in dog 4 (no food and 60 cc. of bile) was 243 cc. for the 24-hour period; with the glucose every six hours and with bile, the output was 208.5 cc. In dog 5 the control output was 224 cc.; the output on glucose was 187 cc. These decreases are significant. It was observed from the hourly readings that in those experiments in which glucose with bile tended to increase the bile output during a 6-hour period, the increase occurred during the first three hours of the period. The decreases were due chiefly to a diminution of secretion during the last three hours of the period. This suggested that glucose tended to increase bile output only during the period of the absorption of bile, and to decrease bile output when bile was not being absorbed (vide ut infra).

When bile was not returned glucose caused a definite decrease in all the dogs. The decrease varied from 20 to 35 per cent. After the withdrawal of glucose, the previous output was not resumed until after six or twelve hours (table 9).

The effect of glucose intravenously. To determine the effect of glucose intravenously three animals were stabilized at a fasting level with and without bile being returned. Then 35 grams of glucose (special C. P.) in 200 cc. of 0.5 per cent NaCl were injected intravenously at the beginning of one or more 6-hour periods; the injection was made during a period of 30 minutes. A 0.5 per cent solution of sodium chloride was used because we desired to control later the effect of the same volume of fluid.

Typical results are shown in table 8. The intravenous injection of glucose increased the output of bile in all of the animals when bile was returned. The first injection markedly increased the output in two animals; in one the maximum increase occurred after the second injection. After the third injection (2 dogs) an increase did not occur; the output was either equal to or less than (dog 3) that of the control level. It was noted from the hourly readings that the increased output with the return of bile did not occur during the period of injection; it occurred during the second and third hour of the 6-hour period; after the third hour the output returned to the control level. This definitely indicates that the glucose stimulated during the period of bile absorption.

When bile was not returned, the intravenous injection of glucose depressed bile output. This depression of output occurred after the first or second injection and persisted for from twelve to eighteen hours after the injections were stopped.

The injection of 200 cc. of 0.5 per cent NaCl solution had no effect on bile output (table 8). This result controls the question of fluid volume. However, the glucose solution was hypertonic (17.5 per cent) and the saline, control solution was slightly hypotonic, and the rate of the injection of glucose was greater than the usual intravenous "glucose tolerance". An isotonic solution of glucose was not chosen and the glucose was not given at a slower rate because it would require a larger volume of fluid and a prolonged rate of injection, factors which we believed to be more objectionable (disturbing the animal decreases bile output) than those we introduced. This result shows, like our results on water drinking (vide ut supra), that a mild tendency toward hydremia does not affect bile output.

The effect of the addition of glucose to a mixed diet on bile volume output. Four animals were stabilized on the mixed diet with the return of bile; then 70 grams of glucose were added to each 6-hour feeding for one or two 6-hour periods. The animals ate this food with their usual excellent appetite. The results were variable. In dog 1 a single feeding of the "glucose-mixed" meal resulted in a 11 per cent decrease in output during the

6-hour period which was followed by a 12 per cent increase during the next 6-hour period and then by the control output. Dog 2 manifested either a similar response, or a 10 per cent increase during the "glucose-mixed" diet, 6-hour period, which was followed by a 10 per cent decrease for the next two 6-hour periods. A third dog was either not influenced or a 10 per cent decrease occurred. The fourth dog manifested only an increase in output of from 8 to 11 per cent. The only conclusion warranted by the results is that the addition of the glucose to the mixed diet with the return of bile slightly altered the output in an unpredictable manner, the effect (an increase or decrease) being reproducible in only one of four dogs.

We selected glucose to study because it is so commonly employed clinically and because the consensus of the opinion in the literature is that bread, among the different foods, excites bile output to a less extent than meat, liver and fatty foods. Cane sugar is reported to have no effect on, or to slightly stimulate, or to inhibit bile output. Our results on glucose, if applicable to man, have certain obvious practical implications.

The most important observation made concerning the influence of glucose on bile volume output is that its influence is conditioned by the presence of bile in the intestine. When glucose alone is given either orally or intravenously, bile output may be decreased from 20 to 35 per cent over a period of from six to eighteen hours. In no instance in numerous tests on four dogs was an increase observed. The decrease may not appear until after the second or third 6-hour administration of the glucose. It is clear from the results of the oral and intravenous administration of glucose that glucose only stimulates during the period of maximum effect of bile on the liver. Like olive oil, the stimulating effect of repeated doses of glucose disappears even when bile is returned. A reasonable explanation of this phenomenon has not occurred to us. Forsgren's observations on the relation of glycogen formation to bile output may apply (8).

Our results on the effect of adding glucose to a mixed diet confirm the variable results reported in the literature on the effect of adding cane sugar to a mixed meal. The effect of the addition of glucose to the meal could not even be predicted in the same dog. Since bile was returned in our experiments, an increase in bile output might have been expected to occur during the early period of digestion. But the amount of glucose entering the intestine probably was inadequate to exert such an effect. As in the case of olive oil, the addition of 70 grams of glucose to the mixed meal would tend to alter the rate of gastric secretion and evacuation. Meat was the only food which when added to a mixed meal produced a definite and significant increase in bile output. This, we believe, demonstrates that the effect of foods on bile output is due to a chemical effect and is not related to their caloric value (9).

The effect of gall bladder bile on the volume output of bile. Although it

might be anticipated that gall bladder bile is a more potent excitant of hepatic bile output than hepatic bile (10), it was deemed advisable to collect data on the question. Four animals were stabilized on a mixed diet and, or a meat diet with the return of 50 or 60 cc. of hepatic bile. Then the hepatic bile was replaced with 20 cc. of gall-bladder bile. The volume of 20 cc. was chosen because it approximated the amount of bile in the gall bladder of the dogs when they were operated. It was found that 20 cc. of gall bladder bile was from 15 to 20 per cent more effective than 50 or 60 cc. of hepatic bile; the effect, however, was not carried over into the following 6-hour period. When 50 cc. of gall-bladder bile were used instead of 20 cc., the increase in bile output amounted to more than 100 per cent; the effect was not more prolonged than one 6-hour period. The larger quantity (50 cc.) of gall bladder bile even when given undiluted at a rate of only 1 cc. per minute tended to provoke emesis.

Incidental observations. Cholagogue action of beef liver. During the course of this study it was found that 100 grams of beef liver yield from 18 to 30 per cent more bile than 125 grams of Pard, or 125 grams of inside beef ham, or beef heart, when all are added to a mixed diet with the return of bile.

Vomiting. Vomiting without exception definitely decreased bile output. This occurred as a result of too rapid eating, a meal that was too large, too large amounts of olive oil, or to the introduction of gall-bladder bile into the intestine. The animals were not ill, because, if permitted, they would reingest the food with apparent appetite.

Psychic secretion of bile. The volume output of bile appeared to be augmented by from $\frac{1}{2}$ to 1 cc. when the animals were teased with food or were permitted to observe the preparation of their food. Since this did not always occur, and since the animals were very active, one could not be certain whether the increase was due to a psychic effect. To settle this point sham-feeding experiments must be performed.

Spontaneous changes in the color of the bile. Since the bile was collected in a graduated glass cylinder, not a balloon, changes in the color of the bile could be observed. These biles of different color, always crystal clear, may form distinct layers in the cylinder. In one instance when the animal was secreting a deep dark golden bile at a rate of 18 to 22 cc. per 6-hour period (no food and no bile), olive oil was fed; the color of the bile was observed to become definitely lighter in color within a ten minute period, a discrete layer being formed; then the bile became darker later, almost as abruptly, without a change in rate of secretion.

Response to dehydrocholic acid intravenously. When a biliary fistula dog is healthy and eating the food ration with relish, the intravenous injection of dehydrocholic acid uniformly augments bile volume output. But if the dog develops distemper, or a hepatitis, as occurred during the

early period of the development of our method, dehydrocholic acid intravenously has no or very little effect on bile volume output. This observation confirms McMaster, Broun and Rous (3).

SUMMARY AND CONCLUSIONS

A method is described wherewith consistent and reproducible results on the bile-volume output of the dog may be obtained under different experimental conditions. The chief principle of the method is the application of continuous suction to the tube in the common bile duct.

The data obtained show that the liver under controlled conditions is as constant in its volume-output of secretion as any of the external secretory glands.

The bile-volume output of seven dogs on a regime of no food and no bile ranged from 6 to 10 cc. per kilo per day. On a mixed diet fed every six hours without the return of bile to the intestine, the output ranged from 13 to 18 cc. per kilo. On the same diet with the return of a standard amount of bile, it ranged from 24 to 27 cc. per kilo per day.

Hot weather has no effect on volume output when the appetite is normal. Water, as it is usually ingested, has no effect on bile volume output.

When a fasted animal not receiving bile is fed a mixed diet or meat, the bile output increases the first and second hours post-cibum and continues at the higher rate during subsequent 6-hour feedings. If bile is returned with the first and subsequent 6-hour feedings, a typical secretory curve is obtained after each feeding, the largest volume being secreted usually during the second hour.

On maintaining the caloric intake of a mixed diet constant, the quantity of bile secreted on a 6-hour feeding schedule is somewhat larger than on a 24-hour single feeding schedule. The maximum output from a single meal occurs during the first six hours, but the effect of the meal is evident for twelve hours.

No difference between the day and night output of bile exists if the dog is fed a mixed diet every six hours during the twenty-four; but if a meal is not fed at midnight the night secretion is less than that of the day. Feeding meat more frequently than every six hours, but maintaining the caloric intake constant, did not augment bile output.

The potency of meat as a stimulant of bile-volume output, which is quite generally agreed to in the literature, is confirmed. The addition of meat to a mixed diet also increases bile output. One hundred grams of beef liver when added to a mixed diet produce from 18 to 20 per cent more bile than 125 grams of beef ham, or beef heart. Yet, per gram weight hepatic bile is considerably more potent than any food. Gall-bladder bile is, of course, more potent than hepatic bile per cubic centimeter.

The ingestion of 20 cc. of olive oil by a fasting dog with or without

the return of bile to the intestine stimulates bile output in most, but not all dogs to the same extent as 125 grams of raw, ground meat. The stimulating effect of olive oil (fed alone) disappears when it is repeatedly ingested at 6-hour intervals; the disappearance of its stimulating effect occurs sooner when bile is not returned to the intestine. When 60 cc. of olive oil are added to a mixed diet, bile output is not stimulated. This is probably due to the effect of the oil on gastric evacuation; this is unlike meat. Thus, olive oil is not a constantly reliable excitant of bile secretion, except when fed once or twice after a period of fasting. *It must be recalled that these conclusions obtain in the absence of the gall bladder.*

When glucose is given orally or intravenously (35 grams) without bile being returned to the intestine, bile output is decreased from 20 to 35 per cent, and the depression of bile output may last for from six to eighteen hours. When glucose is injected intravenously and bile is being absorbed from the intestine, bile output is increased. However, after a third injection, made at 6-hour intervals, bile secretion may be inhibited. When glucose (70 grams) is added to a mixed diet, bile being returned, an unpredictable slight increase or decrease occurs.

Meat and liver are the only foods studied which, when added to a mixed diet, consistently increased bile-volume output.

Vomiting, nausea, anorexia decrease or markedly depress bile output.

Very definite changes in the color of bile secreted may occasionally occur within a period of five or ten minutes.

In the presence of a definite hepatitis, dehydrocholic acid intravenously, or bile introduced into the intestine has no or very little effect on bile volume output (3).

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THE PREVENTION OF ADRENALIN LUNG EDEMA BY THE ALARM REACTION

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In previous publications we described a syndrome characterized by definite morphological changes and alterations in the blood chemistry which may be produced by any damaging agent as long as it is serious enough and acts for a sufficient length of time. Our experiments led us to regard this syndrome as the expression of a general alarm of the organism when suddenly confronted with a critical situation with which it is not prepared to cope. Therefore, we termed it the "alarm reaction" (1–8).

Since the symptoms and signs of this reaction have been described elsewhere (9), we shall not enumerate them here but we wish to call attention to the fact that one of the most striking consequences of an alarm reaction is an increase in the general resistance of the animal, not only to the stimulus with which the reaction has been produced but also to damaging stimuli of a different nature. Thus we showed that rats in which an alarm reaction has been produced by means of formaldehyde injections become more resistant, not only to formaldehyde but also to morphine and the converse is also true since animals pretreated with morphine become more resistant, not only to this drug but also to formaldehyde. Several other examples of such non-specific increase in resistance have been given (10) but in all of these cases the injections have been made subcutaneously and consequently one might object to the conclusion that a true increase in resistance has been obtained because it is conceivable that an animal in the alarm reaction does not absorb the drug as rapidly as a normal one and consequently the actual concentration of the drug in the circulation never reaches toxic levels. Such an assumption would seem all the more reasonable, since in the alarm reaction there is marked tendency towards edema formation which may be considered to be a sign of a disturbance in the peripheral circulation.

Since it is of the greatest importance for the interpretation of the physiological significance of the alarm reaction to ascertain whether it does increase general resistance and therefore, whether it can be regarded as a

non-specific defence reaction against damage as such, we decided to perform further experiments to clarify this point. In order to eliminate the possibility of a delayed absorption of toxic agents from the subcutaneous tissue, we tested the resistance of animals against the intravenous injection of a toxic substance after an alarm reaction had been previously produced in them by various means. Since we had had an opportunity to establish the minimum lethal dose of intravenously injected adrenalin in a large series of rats in our colony, we chose this substance for our experiments.

Previous investigators have shown that the immediate cause of death in most species, following an intravenous injection of adrenalin is usually pulmonary edema (11, 12, 13) and we are able to confirm this on the basis of our experiments in the rat. The question was, therefore, can an alarm reaction prevent the fatal lung edema which usually ensues after the intravenous administration of a lethal dose of adrenalin.

For these experiments, we used 48 hooded black and white female rats, aged 3 months, weighing between 118 and 154 grams with an average weight of 134 grams. They were divided into six groups of 8. In five of these groups, we produced an alarm reaction by various damaging agents, while the sixth group was left as an untreated control.

In the first group, the pretreatment consisted of two subcutaneous injections of 0.4 mgm. of adrenalin in 0.4 cc. of water given at an interval of six hours.

In the second group, the animals were pretreated with three subcutaneous injections of 0.5 cc. of a 4 per cent solution of formaldehyde. These injections were given at regular intervals within 20 hours.

In the third group, the rats were exposed to a temperature of +2°C. for 24 hours.

In the fourth group, the animals were placed in revolving cages, having a diameter of 12 inches and turning at a speed of 18 to 20 revolutions per minute. They were exercised for two hours, then they had a rest of six hours after which they were exercised again for two hours.

In the fifth group, surgical shock was produced by crushing the stomach, the jejunum and the cecum with a hemostat at several places, so as to destroy most of the wall, except the serosa.

Previous experiments have shown that all these damaging stimuli are capable of producing a typical alarm reaction under the experimental conditions described above, and that the symptoms of this reaction will be quite obvious, 20 hours after initiation of the treatment. We therefore proceeded to inject 0.03 mgm. of adrenalin hydrochloride dissolved in 0.3 cc. of water intravenously into all these rats, including the eight not pretreated controls, twenty hours after commencing the treatment of our experimental animals. We found it essential to dissolve the adrenalin

in this small quantity of water, in order to make rapid injection possible, since if the injection is made very slowly, the hormone is less toxic and it is more difficult to obtain comparable results by injecting a larger amount at exactly the same speed than it is when a small quantity is suddenly introduced into the blood stream.

The results of this experiment are summarized in the following table:

ALARM REACTION PRODUCED WITH	NUMBER OF DEATHS IN GROUP OF 8 FOLLOWING INTRAVENOUS INJECTION OF 0.03 MGM. OF ADRENALIN
Adrenalin	0
Exercise	1
Surgical shock	3
Formaldehyde	2
Exposure to cold	2
Not pretreated controls	7

From this experiment, it seems obvious that an alarm reaction increases the resistance to adrenalin. This increase is not only obtained in case the alarm reaction is produced by adrenalin itself, but also when it is elicited by entirely different stimuli.

Since, as we have mentioned, the death following intravenous administration of adrenalin is usually caused by lung edema, it appeared of interest to establish whether an alarm reaction would also prevent the occurrence of an acute pulmonary edema produced by other means.

We found that if bilaterally nephrectomized rats are given large amounts of physiological saline solution intravenously, they develop fatal pulmonary edema within 24 hours. In order to establish whether this pulmonary edema may also be prevented by a stimulus causing an alarm reaction, we performed a bilateral nephrectomy in 12 male rats, 4 months of age, of 200 grams average body weight. Immediately afterwards, all of them received 15 cc. of an 0.9 per cent NaCl solution intravenously. Six of these animals were exercised in a manner similar to that described in the previous experimental series. The remaining six acted as not pretreated controls. Twenty-four hours after the nephrectomy, the controls all showed obvious signs of pulmonary edema, with large amounts of scum oozing out of the mouth and nose, while the exercised group showed no respiratory disturbance. Since at this time, the condition of the not pretreated animals was so bad that it was obvious that they would die before the pretreated ones, we decided to kill all animals at the same time, so that a fair comparison of the appearance of the lung might be possible. At autopsy, we found that only one rat of the exercised group showed traces of edema in one lobe of the lung while all other animals had per-

fectly normal lungs. In the not exercised control group, however, marked lung edema was found in every animal. From these experiments, it appears that an alarm reaction prevents not only the pulmonary edema caused by adrenalin but also that produced in nephrectomized animals.

In previous papers, we have emphasized that the protective action of the alarm reaction is particularly evident against damaging agents which when introduced parenterally have a selective effect on a certain organ. Thus, for instance, we observed that the acute phlegmonous appendicitis produced by the intravenous administration of histamine in experimental animals, is readily prevented, not only by pretreatment with histamine, but also by pretreatment with any other damaging agent capable of producing an alarm reaction (14, 15). We also noted that the acute edema of the face and the paws produced in the rat by parenterally administered egg white may be prevented not only by pretreatment with egg white but also by other "alarming stimuli" (9). It is rather remarkable that during the alarm reaction, animals should be more resistant than otherwise because this reaction is always elicited by inflicting some serious injury upon the organism and the clinical appearance at this time indicates that a condition of serious shock has occurred. We wonder whether one could not explain at least part of this increase in resistance by assuming that the alarm reaction acts by deviating the selective action of drugs on certain organs just as the so called "counter irritation therapy" (blistering, cupping, etc.) or non-specific protein therapy seems to deviate the action of nocuous agents on certain organs.

SUMMARY

It has been shown that an alarm reaction produced by treatment with adrenalin, formaline, exposure to cold, forced exercise or surgical trauma increases the resistance of the rat to the subsequent intravenous injection of a lethal dose of adrenalin. This observation invalidates the possibility that the alarm reaction increases resistance only because it delays the absorption of toxic agents into the blood stream.

Since the immediate cause of death following intravenous injection of adrenalin is pulmonary edema, it seemed of interest to establish whether an edema of the lung elicited by other agents could also be prevented by the alarm reaction. This has been found to be the case, since the pulmonary edema normally caused by bilateral nephrectomy combined with the intravenous injection of large quantities of physiological NaCl solution is also prevented by an alarm reaction produced by exhausting forced exercise.

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THE EFFECT OF BILATERAL ADRENALECTOMY ON ARTERIAL BLOOD PRESSURE OF DOGS WITH EXPERIMENTAL HYPERTENSION

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Clinical evidence proves in some cases and suggests in others that endocrine glands are involved in the mechanism responsible for certain kinds of arterial hypertension in man. Investigation in animals of this phase of the mechanism of hypertension has been made feasible by the discovery of Goldblatt, Lynch, Hanzal, and Summerville (1) that constriction of the renal arteries produces sustained hypertension.

It was shown by Page and Sweet (2, 3) that removal of the hypophysis in hypertensive dogs reduces the level of the blood pressure markedly in those animals in which clinical evidence of reduced endocrine function is prominent. But since increase in intensity of constriction of the renal arteries produces further elevation in blood pressure of these dogs, it is clear that the hypophysis is not alone essential to the mechanism of this type of experimental hypertension. The effect of its removal is to dampen rather than to abolish the results of constricting the renal arteries on the blood vessels. This suggested that the hypophysis acted on the blood vessels indirectly through its well-known stimulating action on other endocrine glands.

Goldblatt reported in February, 1937 (4) that bilateral adrenalectomy eliminates completely the response to constriction of the renal arteries. Further, inactivation of the adrenal medulli does not prevent the rise in pressure which normally follows such constriction. In a more recent communication (5) he found that adrenalectomy also abolishes hypertension once it has been established. Treatment with salt and cortical extract maintains the adrenalectomized animals in apparent health. In some of such animals Goldblatt found a small but definite hypertension could be produced. Abstention from treatment was always followed by a prompt fall in arterial pressure.

The importance of these observations made it appear desirable to repeat them. The experiments reported in this communication were made inde-

pendently of Goldblatt's after the appearance of his preliminary report. They may therefore, be considered independent confirmation of his observations. As this paper goes to press Blalock and Levy (6) report that in 5 experiments bilateral adrenalectomy caused hypertension to disappear within 16 hours.

METHODS. Normal male and female dogs were employed. Blood pressure was measured either by the Van Leersum method or by auscultation over an artery just above the ankle of the hind leg. By the latter method diastolic as well as systolic pressures were obtained. In many cases the pressure was checked by direct intra-arterial measurement. The Goldblatt clamps[1] were applied after exposure of the renal artery through a 10 cm. incision parallel to the spinal column and a few centimeters ventral to it. The incision started just cephalad to the angle made by the last rib with the spine.

A week or more before removal of the adrenal glands the animals were fed 2 to 3 grams of salt and the same amount of sodium citrate. This was in order to accustom the dogs to the altered diet. The salt administration was continued after operation. At one operation the left adrenal gland was removed through a lumbar incision and several weeks later the right one was removed. Every effort was made to secure hemostasis during the operation. Adrenal cortical hormone was administered (1 cc. intramuscularly) on the day of operation and every day thereafter until for reasons of the experiment it was withdrawn.

Four dogs were subjected to the following procedure. The left adrenal gland was removed and the left renal artery clamped. Several weeks later, after hypertension had developed, the right adrenal gland was partially demedullated and the right renal artery clamped. The demedullation was performed by opening the gland with a knife and sucking out as much medulla as possible by means of a sharp glass tube. The gland was then sutured together. All of the medullary tissue is not removed by this method as shown by subsequent histological examination, but certainly it is markedly reduced.

The testes were removed from two hypertensive dogs and the ovaries from two other dogs with hypertension.

Sustained (two months or more) systolic and diastolic hypertension was produced in some of the dogs and then the adrenal glands were removed. In other dogs the clamps were applied to the renal arteries at the operation during which the remaining adrenal gland was removed. In all, 24 dogs have been studied over periods up to 10 months.

[1] We have found it advantageous to have the clamps made of stainless steel rather than silver because it does not corrode or dissolve in the body and is cheaper. From inspection there appears to be less foreign body reaction to the steel than to the silver clamps, but we are not certain of this.

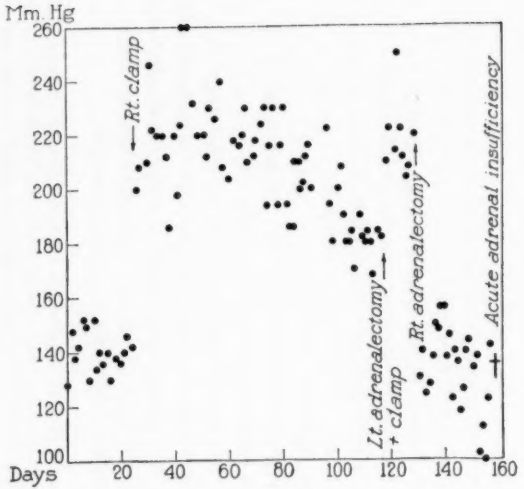

Fig. 1

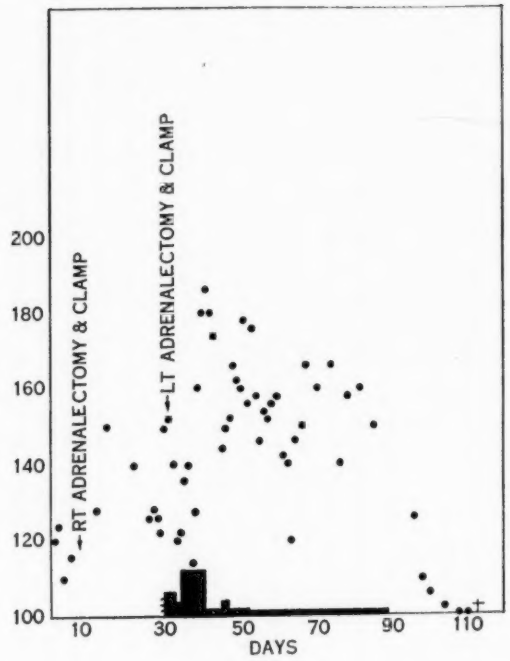

Fig. 2

Fig. 1. Effect of bilateral adrenalectomy on elevated arterial pressure produced by constricting a renal artery.

Fig. 2. Hypertension produced by constricting both renal arteries in a dog with both adrenal glands removed but receiving treatment with cortical extract, salt, and sodium citrate. The animal died with hypotension when cortical extract was withdrawn indicating the completeness of the adrenalectomy.

RESULTS. Removal of one adrenal gland does not appear to alter the hypertension due to constriction of the renal artery (fig. 1) nor prevent its development. If, however, the remaining gland is removed, hypertension is rapidly abolished (fig. 1). In some experiments the hypertension is no longer found when the animal recovers from the anesthetic, while in others several days may be required before the pressure returns to normal. If cortical extract and salt treatment are instituted immediately after operation, the fall is much more gradual (fig. 2).

The usual maintenance dose of cortical extract has been 1 cc. daily along with 3 grams of sodium chloride and 3 grams of sodium citrate. The

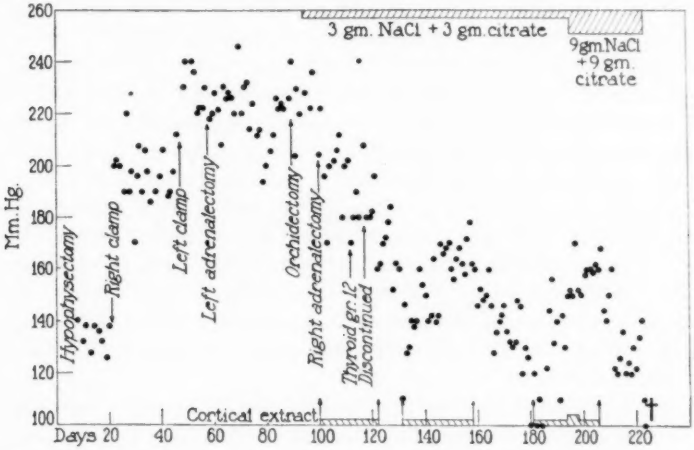

Fig. 3. Effect of the removal of the hypophysis, the testes, and both adrenal glands on arterial hypertension produced by constricting both renal arteries. The hypophysis was removed first, followed by clamping both renal arteries. The left adrenal, both testes and then the right adrenal were removed at separate operations. During intermittent periods cortical extract was administered. Salt and sodium citrate were given daily.

animals appeared to be in excellent condition. Four of them that were kept alive for a four-month period or more developed soft, extremely luxuriant coats similar to those observed following hypophysectomy. If the dose of cortical extract was increased to as much as 6 cc. given in 3 divided doses during the day for 5 to 10 day periods, no corresponding further rise in arterial pressure was observed. With both kidneys clamped and adequate dosage (6 grams) of salt and cortical extract (1 to 2 cc.), definite, though moderate, hypertension was observed (fig. 2). Withdrawal of cortical hormone was invariably followed by a sharp fall in blood pressure to subnormal levels (fig. 3). Institution of treatment was fol-

lowed by a marked rise. Often the rise was not to the original level from which it had fallen.

If adrenalectomy was performed first and then clamps applied to the kidneys without treatment, no rise occurred in the pressure. The experiment was shortly terminated by death of the animal.

Pre- and post-operative treatment insures the life of the animal and constriction of the renal arteries often is followed by a modest rise in pressure. There can be little doubt that the rise in arterial pressure would have been much greater in normal animals than in those with adrenals removed. Experience has taught the extent to which the renal clamp must be tightened to produce marked hypertension. Although the result is not invariable, nevertheless, it occurs in a sufficient number of animals to give one faith in the procedure. When the animals were sacrificed or died from lack of treatment, the extent of the renal constriction was always carefully observed. In most of the experiments a severe rise in pressure might have been anticipated from the extent of the constriction had the animals been normal ones.

Removal of one adrenal gland followed several days later by partial demedullation of the other did not prevent the development of marked hypertension when the renal arteries were constricted (4 experiments). Post-mortem microscopic examination showed the persistence of very small amounts of medullary tissue. Consequently this evidence does not entirely rule out the medulla from participation in the genesis of this type of hypertension.

Hypophysectomy was performed in one dog followed 30 days later by application of both clamps to the renal arteries (fig. 3). A sharp elevation in blood pressure occurred. The left adrenal was now removed. No change in pressure was observed. The testes were then removed; again no change in pressure followed. Cortical extract and salt treatment were begun and the remaining adrenal gland removed. Arterial pressure fell from an average level of 220 to 240 mm. Hg to 190 to 210 mm. within 8 days. Large doses (12 grains) of thyroid substance were administered for 5 days. During this period a sharp but transient rise in pressure occurred. Six days later cortical extract was discontinued. The arterial pressure fell to 110 mm. Hg. The animal began to lose its appetite and moderate diarrhea developed. Resumption of cortical extract was followed within a day by a rise in pressure to 146 mm. Hg. Twenty-eight days later cortical extract was again withdrawn and the arterial pressure again fell, to resume its former level on resumption of treatment. Now cortical extract was withdrawn, but the intake of sodium chloride and sodium citrate was tripled (9 grams of each). Eighteen days later the animal died of adrenal insufficiency with subnormal blood pressure.

Discussion. The results of this investigation which confirm and

amplify those of Goldblatt, show that the adrenal glands and more specifically the adrenal cortex or extracts of the adrenal cortex are a necessary part of the mechanism by which arterial hypertension is produced when the renal arteries are constricted. Other endocrine glands appear to have a modifying influence on the development of hypertension but none so far examined exert so profound an effect as the adrenal cortex. Removal of the hypophysis may dampen the vascular response to renal artery constriction, but it does not prevent it even in the absence of treatment. Removal of the gonads in either male or female dogs (4 experiments) does not appear to modify appreciably the hypertension once it has been established. But removal of the adrenal glands without subsequent treatment abolishes the response completely to constriction of the renal arteries. Treatment with salt and cortical extract restores the response though by no means to that observed in normal animals. In our experiments even very large doses of commercial cortical extract (6 cc. daily) were no more effective than moderate maintenance doses (1 cc.). Nor did increasing the dosage of salt (to 9 grams) and sodium citrate (to 9 grams) increase the response.

Our attempts to destroy the medulla of a remaining adrenal gland were not entirely successful. Histological examination showed that a small amount of medullary tissue persisted or that regeneration had occurred after operation. In such animals hypertension was readily produced. Goldblatt's experiment (1) on dog 8–9 is more convincing. He found that excision of the right adrenal gland, destruction of the medulla, and denervation of the left adrenal did not prevent development of hypertension after the renal arteries were moderately constricted. It appears that the adrenal medulla is not necessary for the genesis of this type of hypertension.

CONCLUSIONS

1. The adrenal cortex plays an important part in the mechanism responsible for development of hypertension in dogs by constricting the renal arteries. The adrenal medulla does not appear essential.

2. Neither the ovaries nor testes are essential for the maintenance of hypertension in dogs with their renal arteries constricted.

3. Administration of maintenance doses of adrenal cortical extract and possibly salt is necessary for persistence of moderate hypertension in dogs with both renal arteries constricted and the hypophysis, testes, and adrenal glands removed.

4. The opinion is offered that endocrine glands in hypertensive animals of this type are concerned chiefly with maintenance of the body in such a state that it can respond to constriction of the renal arteries by development of arterial hypertension.

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DISTRIBUTION OF LACTIC ACID BETWEEN BLOOD AND MUSCLE OF RATS

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Since the finding of Bock et al. (2) that in exercise there is no significant change in alkaline reserve of the blood of a man until a critical metabolic rate is exceeded, there have been many reports showing that lactic acid, a function of alkaline reserve, does not accumulate in the blood below this critical level of metabolism. The literature on this subject has been reviewed by Dill (4).

Not only can moderate work be performed without raising the level of lactic acid in the muscles but also an oxygen debt of about three liters can be contracted independently of lactic acid accumulation These conclusions, reached by Margaria, Edwards and Dill (5, 11, 9, 10), involve the assumption that blood lactate is representative of muscle lactate concentration during work which can be carried on in a steady state. Also, it has been assumed that one to five minutes after severe anaerobic work equilibrium between muscle and blood is established

Recently, Sacks and Sacks (14) have concluded from experiments on anesthetized animals that the existence of an alactacid mechanism for accumulating an oxygen debt as postulated by Margaria is doubtful. Blood plasma issuing from a cat's gastrocnemius muscle that is stimulated at a constant rate contains a much lower concentration of lactic acid than the muscle itself. Thus it may be that in moderate exercise lactic acid is retained in the muscle.

The conflicting conclusions drawn from the studies of man and of isolated muscle led to the plan of the present work. Muscle and blood for determination of lactic acid were obtained simultaneously from rats a few seconds after exercise on a motor-driven treadmill or after swimming in water at 40°C. Non-exercised rats served as controls.

PROCEDURE. Fasting white rats, male and female, weighing roughly 150 grams, were used. The day before the experiment, the hair was removed from one of their hind legs with a depilatory solution. In some instances the rats swam in water at 40°C.; in others they ran on a motor-driven treadmill[1] (table 1). The metabolism of rats running on this

[1] The treadmill was designed by Dr. Gordon C. Ring for work on the metabolism of exercise in rats and is described by him (13). It consists essentially of a box with a moving belt at the bottom, driven by motor. The box can be made air-tight and inserted in a closed circuit apparatus for measuring oxygen consumption of the exercising rat.

TABLE 1

Lactic acid in rat blood and muscle in rest and exercise

Concentrations are in milligram per 100 grams of tissue except on August 17

DATE	REST			EXERCISE								
	Rat no.	Blood lactate	Muscle lactate	Rat no.	Blood lactate	Muscle lactate	Type of exercise	Duration of exercise	Speed	Grade	Work index*	Notes on exercised rats
1937								*mins.*	*meters/min.*	*per cent*		
June 29	1	Lost	38; 42†	4	7	75; 68	Swimming	10				Two cuts to remove leg
June 29	2	11	43; 47†				in water					
June 29	3	8	40; 39†				at 40°C.					
July 2	1	20	40	3	15	37	Swimming	37				
July 2	2	8	41	5	13	66	in water	37				
July 2	4	18	56	6	39	50	at 40°C.	35				Nembutal 0.5 cc. intraperitoneally at end of work
July 20	1	19	20	2	30	Lost	Running on	18	10	89	5	
July 20	6	12	17	3	27	23	motor-	32	10	89	5	
July 20	7	16	24	4	25	30	driven	73	10	89	5	Struggled; stunned with blow on head
July 20				5	43	38	treadmill	47	10	89	5	
July 26	8	17	22	1	80	71	Running on	10	17	100	10	Looked tired
July 26				3	Lost	54	motor-	15	17	75	8	Good runner
July 26				4	68	65	driven	16	17	75	8	Erratic runner
July 26				5	26	58	treadmill	22	17	75	8	Good runner
July 26				6	46	46		7	17	100	10	Fair runner
July 26				7	75	68		25	17	100	10	Fair runner
Aug. 2	3	8	10	1	202	233	Running on	11	17	75	8	Excited, dazed
Aug. 2	7	10	20	2	81	61	motor-	23	17	75	8	Poor runner
Aug. 2				4	42	38	driven	18	17	75	8	Poor runner
Aug. 2				5	52	58	treadmill	23	17	75	8	Poor runner
Aug. 2				6	68	62		30	17	75	8	Poor runner
Aug. 11	1	12	22	2	92	91	Running on	6	43	0	5	Good runner
Aug. 11	8	10	18	3	79	50	motor-	8	20	39	7	Fair runner
Aug. 11	5	16	44	4	126	124	driven	7	21	39	7	
Aug. 11				6	85	76	treadmill	13	20	60	9	Good runner
Aug. 11				7	60	79		6	20	40	7	Fair runner
Oct. 3	1	13	25	2	17	31	Running on	22	12	20	3	
Oct. 3	4	10	19	3	25	23	motor-	28	12	20	3	
Oct. 3	5	12	16	6	16	36	driven	14	12	20	3	
Oct. 3	7	11	23	8	15	25	treadmill	20	12	20	3	
Oct. 3				9	35	79		10	12	20	3	Struggled before being killed
Oct. 3				10	15	35		72	12	20	3	

* The work index shows the approximate increase in metabolic rate over the resting level.

† The lactate of muscle was determined on the supernatant fluid of one extraction and on the total fluid from a series of subsequent extractions of the same tissue. Both values are given.

TABLE 1—*Concluded*

DATE	REST			EXERCISE								
	Rat no.	Plasma lactate	Muscle lactate	Rat no.	Plasma lactate	Muscle lactate	Type of exercise	Duration of exercise	Speed	Grade	Work index*	Notes on exercised rats
1937		*mgm. per 100 gm. H_2O*	*mgm. per 100 gm. H_2O*		*mgm. per 100 gm. H_2O*	*mgm. per 100 gm. H_2O*		*mins.*	*meters/min.*	*per cent*		
Aug. 17‡	7	26	36	1	174	120	Swimming	21				Exhausted
Aug. 17	8	57	35	2	47		in water	37				
Aug. 17				3	50	44	at 40°C.	38				
Aug. 17				4	33	48		42				
Aug. 17				5	29	31		49				
Aug. 17				6	32	29		53				

‡ Note that results for August 17 are calculated on the basis of milligram per 100 grams of H_2O in plasma and in muscle. See table 2 for determinations of H_2O.

treadmill at 14 meters per minute and on a grade of 16 per cent has been found to be raised at least three times the basal level (13). The lowest intensity of work in the present experiments was 12 meters per minute on a grade of 20 per cent.

At the end of exercise, the rat was suspended immediately by two clamps, one attached dorsally to loose skin on the neck, the other to skin on the sacrum. This procedure caused little or no excitement or struggle, the rat being inured to handling. Two persons, each with a pair of sharp shears, then prepared for decapitating the rat and cutting off a hind leg. With the shears in position, a signal was given and the two quick cuts were made simultaneously. The leg, severed at about the mid-shaft of the femur, was instantly plunged into liquid air The time from the end of exercise to the time of cutting was sometimes as long as 60 seconds in the early experiments, but with practice this time was shortened to about 15 seconds. The blood issuing from the neck was collected in a beaker with heparin. The requisite quantity, 1 to 2 cc., was obtained in from 2 to 20 seconds. It was prepared for analysis according to Folin and Wu or, in some cases, was centrifuged near 0°C. and the plasma used. Lactate was determined by the method of Friedemann, Cotonio and Shaffer (6).

From 2 to 5 grams of muscle were obtained from the amputated leg by paring off the skin and separating the bones. The tissue was kept solidly frozen during this dissection by frequent immersion in a mixture of carbon dioxide snow and ether. The frozen pieces of muscle, free from bone and skin, were quickly weighed in a tared Pyrex test tube, 1.5 × 15 cm., and immediately covered with 10 cc. of either 5 or 10 per cent ice-cold trichloracetic acid. Either dilution gave equally complete recovery of lactic acid. The apparatus used for mincing the muscle is described by Potter

and Elvehjem (12). It consists essentially of a revolving cylindrical pestle ground to fit closely into the Pyrex tube. We are indebted to R. E. Johnson of this laboratory for calling our attention to the applicability of this apparatus for grinding muscle tissue. Centrifugation yielded a clear extract, an aliquot of which was treated with copper-lime for removing sugars. This was centrifuged and an aliquot of the supernatant fluid used for analysis. The solutions were kept near 0°C. throughout.

In order to make sure that analysis of an aliquot of the first extract gave dependable results, a series of extractions was made in the experiment of June 29, and lactic acid was determined on the combined extracts. The lactate concentration in muscle determined in this way agreed moderately well with the value calculated from an aliquot of the original supernatant fluid (table 1).

TABLE 2

Water content of plasma and muscle in experiment of August 17

RAT NO.	PLASMA	MUSCLE
	gm. H_2O per 100 gm. plasma	*gm. H_2O per 100 gm. muscle*
1	91.6	78.2
2	93.7*	77.6
3	91.6	78.5
4	94.4*	77.5
5	91.8	78.6
6	94.6	78.1
7	91.2	77.4
8	91.6	77.4

* Calculated from refractive index of plasma. Other values determined by drying at 110°C. to constant weight.

We have been concerned principally in these experiments with the question as to whether an approximate equilibrium is reached between muscle and blood, and for this purpose the difference in water contents and the distribution of lactic acid between cellular and extracellular fluid may be neglected. In one series, however, an attempt was made to obtain a more precise measure of this equilibrium by using blood plasma and calculating the concentration of lactic acid in the water of plasma and of muscle. Shortly after the usual procedure of decapitation and amputation of one hind leg, the opposite leg was severed, frozen, and its muscle separated as usual. Water determinations made on plasma and muscle by drying to constant weight at 110°C. are given in table 2. Although the tissue used for analysis was free from skin and bone, there was included a small proportion of fascia, tendon, and connective tissue. Notwithstanding this and the variability in anatomical boundaries, the water contents of the tissue samples from 8 rats fell within the narrow range of 77.4 to 78.6 per cent.

RESULTS. A summary of the procedure and results is presented in table 1. Blood and muscle lactate in rest and in exercise are given for each dated series. The type of exercise as well as the duration, speed, and grade of running are given. Work done by the individual rats in swimming or running is not adequately described by the information given in table 1. The variation in emotional response and in skill have considerable influence on the amount of work done, particularly at the greater rates of running. It would have been insructive to have had measurements of metabolism in each case, but even this would not have been wholly satisfactory because some of the rats failed to maintain a steady rate of work. The index to work output shown in table 1 was developed from Lusk's

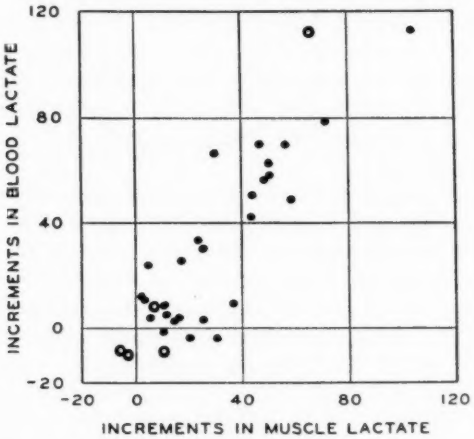

Fig. 1. Increments of lactic acid in blood and muscle. The hollow circles correspond to milligram per cent of lactic acid in plasma water and in muscle water. The other points correspond to milligram per cent in blood and in muscle.

measurements of the energy requirement for horizontal and vertical movement of various animals (8), from Ring's observations on rats (13) and from a few measurements of our own made with Ring's apparatus and his assistance.

Figure 1 is constructed in order to show the relation between lactic acid concentration in muscle and in blood. The points correspond to increments over the average of the control values in the same series. The points fall near a line which passes through the origin and has a slope of unity; that is, the increase in blood lactate is approximately equal to increase in muscle lactate. Some of the values are negative because the concentration of lactic acid was occasionally lower in exercised rats than in the corresponding non-exercised animals.

Interpretation. Before the significance of the relation found between blood and muscle lactic acid in exercise (fig. 1) can be evaluated, we must see whether the values obtained represent the actual conditions of exercise. It is plain that in some cases a slight increase in lactate occurred in both muscle and blood due to the decapitation and amputation. Davenport and Davenport (3) have found that when tissue is obtained from rats at rest without great muscular spasm, blood contains 10 to 15 mgm. per cent and muscle 15 to 27 mgm. per cent of lactic acid. In table 1 it will be noted that in the first two series the six control values for muscle are consistently about 40 mgm. per cent However, with improvement in technique and more dispatch in handling the material, the 15 control values thereafter average 22 mgm. per cent, with a mean deviation of $\pm$ 4. The 18 control bloods average 13 mgm. per cent, with a mean deviation of $\pm$ 3 mgm. per cent. Thus with experience we were able to obtain blood and muscle without increasing their lactic acid content. Our results do not agree with those of Jokl (7), who reported that the concentration of lactic acid in muscle was from 4 to 6 times higher than in blood of resting animals killed without an anesthetic.

As suggested above, we are led to the conclusion that there is a close relation between the increment of lactic acid in muscle during exercise and that in blood. Blood lactate is an index of muscle lactate. These findings do not agree with the conclusions of Sacks and Sacks. Using anesthetized cats and rabbits, with single isolated muscles stimulated electrically at a constant rate, they found that the muscle contained 5 to 15 times the lactic acid concentration of the venous plasma flowing from it. They conclude that in moderate work, failure to observe an increase in blood lactate may be ascribed to its accumulation in the muscle. We have presented evidence to the contrary: in the intact animal muscle lactate parallels the blood lactate at all levels, provided a few minutes are allowed for an equilibrium to be established.

It must be remembered that the exercise was of such intensity that the animals could carry on for 6 to 73 minutes, which time allowed amply for distribution of lactate through the body. In fact, 6 to 8 minutes proved ample time for the distribution of lactate in the hardest grade of work on the treadmill, as shown by the series of August 11, table 1, even though the hind leg muscles used for analysis shared a large proportion of the body work. The muscle was obtained from these rats after from 22 to 40 seconds of recovery, so that lactate removal in the muscle in the payment of oxygen debt is small.

Since much emphasis has been placed upon the fact that moderate work can be carried on by man with little or no increase in blood lactate, particular interest attaches to the experiments on moderate rates of work. Two low rates were chosen; one series of six rats ran at 12 meters per min-

ute on a 20 per cent grade, the other series of 4 rats at 10 meters per minute on an 89 per cent grade. As shown in the table, these conditions cause at least a three-fold increase in metabolism. The average increase of blood and muscle lactate of the exercising rats over the resting value was calculated for each series. For the former series, blood averaged 6 mgm./100 g. increase, muscle 9 mgm./100 g.; for the latter, blood 15 mgm./100 g., muscle 10 mgm./100 g. increase.

Thus work at a metabolic rate at three times the basal level results in little or in some cases no increase in lactic acid in blood or in muscle. We feel entirely justified, therefore, in holding to the opinion that work in man which does not raise the lactic acid level in the blood does not raise it in the muscle. Furthermore, the proposal of Margaria that the oxygen debt contracted at the beginning of such work depends on an alactacid mechanism is validated.

We do not wish to imply that there is complete equilibrium between muscle cells, the site of lactic acid production, and blood plasma. Lactic acid must pass from cells to extracellular fluid and from there into the plasma by a process of diffusion, the gradient for which remains unknown. Very likely the gradient depends on the circulation. The free flow of blood in the intact animal evidently leads to the rapid distribution of lactic acid throughout the body, while the conditions set up by Sacks and Sacks appear to have prevented the free movement of lactic acid from muscle to blood. Our experiment of August 17 in which a longer time was employed for bleeding in order to secure plasma enough for analysis suggests that very nearly complete equilibrium is reached between muscle and plasma. Unfortunately, this experiment is not wholly satisfactory because of the lengthened time required for bleeding; the lactic acid in plasma may have been too high on that account.

Sacks and Sacks found that stimulation of a group of muscles at a constant rate through the same nerve gave different lactate concentrations in different muscles. This raises the question whether the values for muscle lactate in the present work represent an average of widely different concentrations in the many leg muscles represented in each analysis. The facts shown in figure 1 indicate that after six minutes of work, whether hard or easy, large gradients of lactate between muscle and blood do not exist. If diffusion of lactate from muscle to blood brings about this adjustment so rapidly, it seems unlikely that there are large inequalities in lactate concentration among different muscles. Barr and Himwich (1), comparing arterial and venous blood after severe exercise, came to the conclusion that lactic acid is rapidly removed from blood in its passage through less active tissues. This idea is supported by our conclusion that great dissimilarities in concentration of such a freely diffusible substance as lactic acid cannot long exist in an intact animal with unimpeded blood flow.

SUMMARY

Lactic acid has been determined in muscle and blood obtained simultaneously from rats in rest or after exercise of varying intensity. With experience, the procedure used gave the lactic acid concentration of blood in rest as 13 mgm. per cent and of muscle as 22 mgm. per cent.

At the end of all grades of exercise lasting 6 to 73 minutes, the increment in lactic acid concentration in blood and that in leg muscle are approximately equal. Thus in the intact, exercising animal there is free and rapid diffusion of lactate between blood and muscle. The assumption that lactic acid in blood reflects its concentration in muscle is approximately true under these conditions.

Moderate work involves little or no accumulation of lactic acid in muscle or blood. This is confirmatory of the theory that in exercise not involving an increase in blood lactate there is a work mechanism not involving lactate accumulation in the muscle. There may be an oxygen debt that does not depend on lactic acid accumulation.

Conclusions based upon a study of lactate retention in a single isolated working muscle or groups of muscles of an anesthetized animal cannot be applied to the intact animal without reservation.

Acknowledgment. The problem and the method of study were suggested to the author by Prof. D. B. Dill. Invaluable advice in technique was given by Dr. R. E. Johnson of the Fatigue Laboratory and by Dr. G. C. Ring of the Department of Physiology, Harvard Medical School.

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THE EFFECT OF THYROIDECTOMY UPON PANCREATIC DIABETES IN THE CAT

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The effects of thyroidectomy upon experimental pancreatic diabetes have been studied by many investigators. The results have been widely divergent, ranging from reports of the complete suppression of glycosuria to the opposite claims that thyroidectomy does not influence the glycosuria at all. In the hope of clarifying such discrepancies we have prepared a larger series of thyroidectomized-depancreatized animals than has been hitherto reported from a single laboratory.

The available literature on thyroidectomy and pancreatectomy has been fully reviewed. The majority of the papers are cited in the articles of Allen (1), Yriart (2) and Wilder (3). In addition, the work of Baranoff (4) and Shpiner and Soskin (5) should be mentioned. The procedures used included partial or complete pancreatectomy, partial or complete thyroidectomy and ligation of the thyroid arteries. From the original articles we have collected references to more than 30 animals (mostly dogs) which had been studied in an effort to determine the effect of decreased thyroid function upon pancreatic diabetes. The results in almost half of these animals have been interpreted by the original authors as indicating no effect, while the results in the remaining animals indicated a decrease in the severity of the diabetic manifestations. However, the majority of workers are included in the latter group.

Other references to the metabolic rate, clinical analogies, and so forth, will be noted later.

EXPERIMENTAL METHODS. The urinary excretion of glucose, nitrogen and acetone bodies of fasting cats was studied in the following groups: A, 6 depancreatized cats; B, 10 cats from which a piece of muscle from the anterior portion of the neck was removed at the time of pancreatectomy; C, 6 cats thyroidectomized 4 to 34 days prior to pancreatectomy; D, 13 cats from which the thyroid was removed at the same time as the pancreas. When possible the parathyroids were dissected free from the upper pole and replaced in the space left by removal of the thyroid. None of the cats were noted to have symptoms of tetany during the experimental

periods although some of the thyroidectomized cats (C) did so before removal of the pancreas. Some of this group received calcium salts and vitamin D in addition to their regular preoperative diet. All the cats were examined post-mortem and all suspected thyroid or pancreatic tissue was subjected to histological investigation. The animals included in this series were free of thyroid and pancreatic tissue by this examination. Intraperitoneal nembutal (pentobarbital sodium) in a dosage of 30 to 50 mgm/kilo of body weight, was used for anesthesia.

The cats were fasted after pancreatectomy. Urine was collected from metabolism cages in the usual manner with toluol as a preservative. Nitrogen was determined by the micro Kjeldahl method, glucose by Benedict's method and acetone bodies by the technic described by Van Slyke.

In our calculations the first post-operative day commences at 9 a.m. on the day following operation. If no post-operative urine was present on that morning and none could be obtained by pressure over the cat's bladder, the next morning's urine was considered to represent a day and a half instead of a day. Only the first three post-operative days are considered. The weight at pancreatectomy is used to determine the excretion per kilogram of body weight. A few of the animals were killed before the third post-operative day was complete in order that certain other studies might be performed.

Results. Comparison of the control and thyroidectomized groups.

Control group (A + B). It will be noted in table 1 that (A), a depancreatized group, and (B), a group composed of cats which had a small piece of neck muscle removed at the same time as pancreatectomy was performed serve as controls. The difference in the means of these two groups is not statistically significant. It will be noted that the unusually high mean values for group A are in a large part due to a single cat (A-1). They were combined and the group (A + B) used as controls.

Thyroidectomized groups. Group C. Table 1 shows that the difference between the mean values for glucose and nitrogen excretion of the control group (A + B) and the cats thyroidectomized 4 to 34 days before pancreatectomy (group C) is significant. The mean glucose excretion is less by 23.7 per cent $\pm$7.4 per cent and the nitrogen excretion by 20.6 per cent $\pm$8.1 per cent than that of the control group. However, despite the large difference in the means for the ketone values, the decrease is not statistically significant.

That weight loss and malnutrition are not important factors in this decreased excretion of glucose and nitrogen is indicated by the fact that the weight changes of the cats in group C between the removal of the thyroid and the extirpation of the pancreas were -0.09, 0.00, $+0.50$, $+0.50$, -0.30 and -0.15 kgm. respectively in the order listed in table 1. The time in days between thyroidectomy and pancreatectomy was 7, 5, 19, 13,

TABLE 1

Thyroidectomized-depancreatized cats

CAT NO.	SEX	WEIGHT*	AVERAGE DAILY URINARY EXCRETION FOR FIRST THREE POST-OPERATIVE DAYS (FASTING)			
			Glucose	Nitrogen	Ketones	D/N
Group A. Depancreatized only						
		kgm.	*gm./kgm./day*	*gm./kgm./day*	*mg./kgm./day*	
1	F	1.95	5.11	2.03	255.0	2.51
2	M	1.99	3.21	1.35	293.1	2.38
3	F	2.20	3.08	1.45	81.0	2.12
4		2.90	2.77	1.01	26.8	2.74
5	F	3.35	3.97	1.70	177.3	2.34
6	M	3.40	2.96	1.23	85.9	2.41
Mean (A) 6 cats.....		2.63	3.52±0.36†	1.46±0.15	153.2±43.3	2.41
Group B. Depancreatized and piece of neck muscle removed at same time						
1	F	1.37	2.45	1.38	0	1.78
2	M	1.74	2.61	1.41	0	1.85
3	F	2.22	3.40	1.29	316.5	2.64
4	F	2.35	4.39	1.24	167.0	3.54
5	F	2.50	3.33	1.13	44.7	2.95
6	M	2.62	2.57	1.30	0	1.98
7	M	3.00	2.54	1.17	107.0	2.17
8	M	3.03	3.52	1.07	115.3	3.29
9	M	3.87	1.85	0.83	46.1	2.23
10	M	3.99	3.60	1.50	44.4	2.40
Mean (B) 10 cats.....		2.67	3.03±0.24	1.23±0.06	84.1±31.2	2.46
Total Control Group—16 cats (Group A and Group B combined)						
Mean (A + B).....		2.66	3.21±0.20	1.31±0.07	111±26	2.45
Group C. Thyroidectomized 4 to 34 days before pancreatectomy						
1		2.10	2.49	1.00	115.7	2.49
2	F	2.20	2.88	1.31	1.0	2.20
3	M	2.40	2.63	0.93	159.9	2.83
4	M	2.49	2.22	0.87	22.9	2.55
5	F	2.80	1.97	1.28	29.8	1.54
6	M	3.45	2.53	0.87	10.8	2.95
Mean (C) 6 cats.....		2.57	2.45±0.13	1.04±0.08	56.7±26.6	2.43
Percentile decrease from mean of control group (A + B).....			23.7%	20.6%	[48.9%]‡	
Standard error of decrease.....			±7.4%	±8.1%	±33.5%	

* Weight day of pancreatectomy.

† Indicates standard error of the mean.

‡ Brackets [ ] indicate those differences that are not deemed significant since there is more than 5 per cent probability of their arising from a sampling error.

TABLE 1—*Concluded*

CAT NO.	SEX	WEIGHT*	AVERAGE DAILY URINARY EXCRETION FOR FIRST THREE POST-OPERATIVE DAYS (FASTING)			
			Glucose	Nitrogen	Ketones	D/N
Group D. Depancreatized and thyroidectomized at same time						
		kgm.	*gm./kgm./day*	*gm./kgm./day*	*mg./kgm./day*	
1	M	1.32	2.32	1.24	79.1	1.87
2	F	1.70	3.28	1.06	264.0	3.04
3	F	1.72	4.85	1.05	144.0	4.63
4	F	1.90	1.91	0.87	3.9	2.19
5	M	1.94	2.40	0.69	45.8	3.48
6	F	2.75	2.26	1.27	3.1	1.78
7	F	2.97	3.10	1.15	144.2	2.69
8	F	3.00	2.70	1.10	34.8	2.45
9	F	3.05	4.80	1.58	13.1	3.04
10	F	3.22	2.66	1.19	31.4	2.23
11	F	3.35	0.91	0.82	6.2	1.11
12	F	3.62	1.36	0.63	0	2.16
13	M	3.94	2.61	1.07	59.6	2.45
Mean (D) 13 cats.....		2.98	2.70±0.32	1.06±0.07	63.8±21.6	2.55
Percentile decrease from mean of control group (A + B).....			[15.9%]	19.1%	[42.5%]	
Standard error of decrease.....			±11.8%	±7.6%	±30.4%	

4, 34 in the order listed. A tendency to a decrease in nitrogen excretion with the increase in the interval between operations may be noted.

Group D. In the case of cats thyroidectomized at the time of pancreatectomy the difference in the mean values for nitrogen excretion from that of the control group (A + B) is significant, while the difference for glucose and acetone bodies is not. The mean nitrogen excretion is less by 19.1 per cent ±7.6 per cent than that of the control group (A + B).

Discussion. It appears from the analysis of the results that the decreased nitrogen and glucose excretion of the cats thyroidectomized before pancreatectomy (C) is a true difference, which may be attributed to thyroidectomy. In the simultaneously operated group (D), the reduction of glucose and nitrogen is still observed, but the change in glucose falls outside the range of statistical significance. Due to the wide range of acetone body excretion in the control and experimental series, the interpretation is more difficult. In spite of its lack of mathematical validity, the diminution of ketonuria may have a physiological meaning. In general, our results agree with those of the writers who have reported a moderate decrease in glycosuria in thyroidectomized-depancreatized animals as compared to controls.

The variation in the findings described in the literature has occurred, at least to some extent, among the animals of our series. The following factors appear worthy of mention in this connection:

1. Species. The cat is known to have a very acute type of diabetes. Such a severe metabolic disturbance might be less easily modified than the diabetes of the dog, for example. However, hypophysectomy appears to be equally effective in these two species.

2. Marine (6) has stated that aberrant thyroid tissue may be demonstrated in more than 90 per cent of cats and dogs. Disregarding all other factors, the possibility of accessory thyroid tissue might explain some of the differences reported.

3. Weight loss and malnutrition are possible factors causing a diminished glucose and nitrogen excretion in such studies, but have been inconspicuous in the present study.

The fact that thyroidectomy may not be anatomically complete in experimental animals must be viewed in relation to the effects of clinical surgery, usually partial thyroidectomy, in human diabetes. Wilder et al. (3) have shown that thyroidectomy produces an amelioration of the manifestations of diabetes in man. It is well known that any degree of diabetes associated with thyrotoxicosis is improved by subtotal thyroidectomy. Spontaneous myxedema may have the same effect (Shepardson and Wever, 7). Thyroid substance has aggravated diabetes in humans (Falta et al., 8). In like manner thyroid substance has increased the severity of diabetes in partially depancreatized dogs (1) (2) (4).

The usual explanation for the clinical results is that the metabolic rate is lowered by thyroidectomy and the burden on the pancreas and liver reduced. This situation must be compared with experimental diabetes in which the pancreas is removed. In the thyroidectomized animal the metabolic rate does not rise to as high a level after pancreatectomy as it does in the depancreatized controls (Dann et al., 9; Ring and Hampel, 10). In contrast to the effect of thyroidectomy the influence of hypophysectomy on experimental diabetes is marked. However, in the Houssay animal (11) the metabolic rate is essentially the same as in the thyroidectomized-depancreatized animal (9), but the modification of the diabetes is very striking. This would indicate that the thyroid plays a relatively small part in the production of the Houssay type of diabetes.

SUMMARY

1. The effect of thyroidectomy upon the urinary excretion of glucose, nitrogen and acetone bodies of fasting, depancreatized cats during the first three days after pancreatectomy has been studied.

2. Compared with the control group of 16 depancreatized cats (A + B) the animals thyroidectomized before pancreatectomy (C) showed a dim-

inution of glucose and nitrogen excretion of more than 20 per cent which was statistically significant. A diminished acetone body excretion occurred but it was not demonstrably significant.

3. Thirteen cats thyroidectomized and depancreatized simultaneously also showed a decrease in the glucose, nitrogen and acetone body excretion compared to the controls. In this group, however, only the diminution in nitrogen excretion was statistically significant.

4. It appears at present, as Houssay has suggested, that the thyroid plays a relatively small part in the modification of pancreatic diabetes which follows hypophysectomy or adrenalectomy.

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THE ENDOGENOUS NITROGEN AND BASAL ENERGY METABOLISM RELATIONSHIPS IN HYPOPHYSECTOMIZED RATS[1]

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Since removal of the pituitary gland causes the interruption of growth, it becomes possible by such a procedure to study the maintenance requirements of immature animals without the complicating factor of growth entering in. The maintenance requirements for nitrogen and energy under such conditions have been the subject of the present study.

Braier and Morea (1935) report that hypophysectomized rats excrete 20.5 (±4.1) mgm. urinary nitrogen per 100 grams body weight after six days on a N-free diet while normal rats, under the same conditions, excrete 28.3 (±3.9) mgm. Schaffer and Lee (1935) report a nitrogen content 22 per cent less in the bodies of hypophysectomized rats than that of their control litter mates paired to the same food consumption. The latter observation would indicate a greater rate of nitrogen excretion by the hypophysectomized rats. Schaffer and Lee reconcile their findings with those of Braier and Morea by assuming that there is at first an increased proteolytic activity which finally diminishes to make possible the lowered endogenous catabolism reported by the South American workers. The work of Lee and Ayres (1936) again shows that hypophysectomized rats lose specifically more nitrogen than their controls and the results of Perla and Sandberg (1936) also are inconsistent with the hypothesis supported by the work of Braier and Morea. No one, to our knowledge however, has made a direct attempt to confirm or refute the results of the South American workers.

Several investigators have reported finding a lowered basal metabolic rate in hypophysectomized rats. Foster and Smith (1926) found that hypophysectomy lowered the basal metabolic rate from an average of 4.8 Calories per kilogram per hour to an average of 3.2 Calories per kilogram per hour. This is about 67 per cent of normal. Fisher and Pencharz (1936) report that hypophysectomized rats show basal metabolic rates 70 per cent of those of normal controls.

[1] The expenses of this investigation were defrayed by an Alexander Brown Coxe Fellowship Grant.

[2] Alexander Brown Coxe Fellow, 1936–37.

EXPERIMENTAL. All rats studied were males and they were kept on a stock ration (calf meal + dried yeast, ad lib.) when not given the low or protein-free diets.

Hypophysectomies were made when the rats weighed about 100 grams. We wish to thank Dr. Kenneth W. Thompson[3] for performing the operations. A total of nineteen hypophysectomized rats were studied. Some of them were carried through several experiments. Thyroidectomies were made on four additional rats. At least three weeks were allowed after the operations before our experiments were started. Growth had completely ceased during this time yet the animals appeared perfectly normal and seemed to retain their youthful appearances throughout the experiments.

The procedure for measuring the relationship between endogenous nitrogen and basal Calories was as follows: after a fast of 16 to 20 hours the heat production was measured in a Haldane gravimetric metabolism outfit for a period of six hours. The R.Q. was determined for the entire six hours although hourly measurements of the CO_2 production were made. An average of the three lowest hourly records for CO_2 production together with the R.Q. was used to calculate the basal heat production. Immediately following the basal heat determinations the rats were given the low or protein-free diets in collection cages which consisted of four-liter beakers (Ashworth, 1935). The urine was collected in two day periods until the animals were believed to be excreting a minimum of nitrogen or, in the case of the hypophysectomized rats, until they appeared likely to go into hypoglycemic shock. They were then returned to the calf meal diet and allowed to regain their normal weight and vitality after which the same procedure was repeated.

RESULTS. There seems to be little doubt that hypophysectomy lowers the basal metabolic rate of rats. By correlating 32 measurements of the basal heat production of hypophysectomized rats with their body weight the following equation was secured:

$$\text{Calories/day} = 0.467 \text{ body weight}^{0.634}$$

When compared with an equation derived from the data of over 100 normal rats

$$\text{Calories/day} = 0.540 \text{ body weight}^{0.720}$$

the hypophysectomized rats were found to have a basal heat production which averaged only about 57 per cent of normal.

Hypophysectomized rats have delicate appetites and go into hypoglycemic shock readily. It was necessary to try several low nitrogen diets in order to find the one most suitable. The composition of these diets is shown in table 1. Four normal rats and four hypophysectomized were

[3] Fellow in Experimental Endocrinology, 1936–37.

each given a trial on the N-poor diet of table 1. The results may be summarized with the averages shown in table 2. From table 2 it may be readily observed that, while the normal rats had a distinct positive nitrogen balance, the hypophysectomized rats had a slightly negative balance. Apparently the hypophysectomized rats did not utilize the

TABLE 1

Low N diets given hypophysectomized rats

	N POOR	N FREE #1	9 PER CENT YEAST	6 PER CENT YEAST	N FREE #2
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Dextrin	45.5	56	36	50	50
Sucrose	10	10	10	10	25
Salts	4	4	4	4	4
Whole milk pwd	10				
Vitab yeast conc	2.5	2			
Dried yeast			9	6	
Butter	25	25			
Crisco			24	27	18
Cod liver oil	3	3	3	3	3
Karo			14		
N content mgm./gram	6.1	1.8	5.9	4.0	0

Whole milk pwd ..... 39.0 mgm. N/gram
Vitab ..... 72.0 mgm. N/gram
Dried yeast ..... 66.0 mgm. N/gram
Butter ..... 1.6 mgm. N/gram
Ryzamin liver 343 ..... 9.8 mgm. N daily

TABLE 2

CONDITION OF ANIMAL	NORMAL		HYPOPHYSECTOMIZED	
Days on diet	6	10	6	11
Average body weight (grams)	69.0	68.5	93.5	91.3
N intake (mgm./100 grams)	61.0	48.2	28.9	24.1
Urinary N (mgm./100 grams)	30.4	27.4	34.6	24.3
Net N balance (mgm./100 grams)	+30.6	+20.8	−5.7	−0.2

nitrogen of the N-poor diet very efficiently. The four hypophysectomized rats were given a N-free diet (table 1, no. 1) after 13 days of the N-poor diet. Their urinary nitrogen excretion then dropped to an average of 19.7 mgm./100 grams during the next five days. After 18 days on the low nitrogen diet the animals had become quite weak, began to refuse their food and threatened to go into hypoglycemic shock. They were then fed the stock diet. Two other hypophysectomized rats

given the N-free diet without a preliminary period on the N-poor ration showed evidence of hypoglycemic shock after only eight days and while their nitrogen excretion was still high (29.0 mgm./100 gm.).

The diet containing 9 per cent yeast did not prove very satisfactory as a low nitrogen diet for hypophysectomized rats. The animals developed a diarrhea and showed a high excretion of endogenous urinary nitrogen. Lowering of the yeast content of the diet to 6 per cent lowered the endogenous nitrogen excretion slightly but did not prevent the animals from going into hypoglycemic shock.

Two rats given the 6 per cent yeast diet deserve special attention. One of these went into hypoglycemic shock on the seventh day of feeding the diet. It was given glucose intraperitoneally and was revived for a few hours but eventually died without regaining an appetite. The other rat threatened to go into hypoglycemic shock on the eighth day and was given ½ cc. of a crude pituitary extract subcutaneously on the eighth and ninth days. The animal was then continued on the 6 per cent yeast diet for seven additional days and still appeared to be in fair condition. The nitrogen excretion of this animal had leveled off at about 23 mgm. per 100 grams body weight.

All of the low nitrogen diets used thus far had contained the vitamin B complex incorporated in the diet. No additional supplements were fed. In an effort to improve the appetites of the hypophysectomized rats N-free diet no. 2 was prepared and fed with a supplement of 100 mgm. daily each of Ryzamin B[4] and liver extract.[5] The rats were thus given 9.8 mgm. of nitrogen (by analysis) daily from the vitamin supplements and no appreciable amount from the diet. Rats that refused the supplement were fed it by means of a medicine dropper.

Under such a regime hypophysectomized rats survived very well, in fact two of them were kept on the diet 56 days and several survived 40 days. Their urinary nitrogen excretion dropped from an average of 36 mgm. of nitrogen per 100 grams body weight on the second day of the diet to a minimum level of about 20 mgm. per 100 grams on the tenth day. This level was maintained until the end of the experiment. Normal controls under the same experimental conditions excreted an average of 42 mgm. nitrogen per 100 grams body weight on the second day which was 6 mgm. more than their hypophysectomized litter mates. The normal controls continued to excrete more nitrogen until the fifteenth experimental day. It is quite likely that the bodies of the normal rats contained a greater proportion of storage protein at the start of the experiment, thus accounting for the greater excretion of nitrogen at that time.

[4] Obtained from Burroughs Wellcome & Co., Tuckahoe, New York.

[5] Number 343, kindly furnished by the Lilly Research Laboratories, Indianapolis, Indiana.

Braier and Morea's observations (loc. cit.) that hypophysectomized rats excrete less urinary endogenous nitrogen than normal controls would seem to agree with our results on the sixth day. However we believe that their differences too may be explained by a greater content of storage protein in the bodies of their normal rats. If they had carried their experiment for a longer time they would no doubt have confirmed our observations that hypophysectomized rats excrete approximately the same amount of nitrogen as do normal controls.

A primary object of our investigation was to determine what effect hypophysectomy might have on the ratio of endogenous nitrogen excretion to basal metabolism. It has been recently reported that the above ratio remains constant regardless of the species (Brody, Procter and Ashworth, 1934; Smuts, 1935). Two milligrams of endogenous urinary nitrogen are supposed to be excreted for every Calorie of basal heat produced. From determinations on the same rats of both the urinary nitrogen excretion

TABLE 3

	HYPOPHYSECTOMIZED ANIMALS	THYROIDECTOMIZED ANIMALS	NORMAL ANIMALS
No. of determinations	27.00	13.00	130.00
Cal./100 grams	8.52	11.9	14.9
Mgm. N/100 grams	27.5	29.0	23.0
N/Cal. ratio	3.23	2.44	1.55

and the basal heat production we found the averages for rats weighing between 50 and 150 grams to run as indicated in table 3. The nitrogen determinations were made after six days on a low nitrogen diet and are probably not strictly endogenous values. It is probable that the hypophysectomized rats had more nearly reached their endogenous level. The differences in the ratios shown above are practically all due to differences in the basal metabolic rate rather than to any differences in the endogenous nitrogen excretion. They would seem to indicate that the pituitary has a profound influence on the basal metabolic rate and practically no influence on the endogenous nitrogen excretion. From this it would follow that there is no fundamentally constant relationship between the minimum requirements for nitrogen and energy.

SUMMARY

Hypophysectomy causes a marked reduction in the basal metabolic rate of 100 gram rats, to about 60 per cent of normal. It has no appreciable effect on the endogenous nitrogen excretion. Hypophysectomized rats, it is believed, have a lower content of storage protein than do their normal controls.

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COMPARATIVE EFFECTS OF IODOACETATE AND IODOACETAMIDE ON THE OXYGEN CONSUMPTION AND GLYCOLYSIS OF FROG MUSCLE

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It is well established that iodoacetamide reacts considerably more rapidly under physiological conditions of temperature and pH with sulfhydryl compounds of known composition than iodoacetate (Smythe, 1936; Hellström, 1934). On the other hand the physiological order of effectiveness of these two compounds as measured by their rates of inhibition of fermentation by living yeast and yeast preparations is the reverse of that found *in vitro* for the reaction with —SH groups (Smythe, 1936). These results are of considerable interest since, after the reaction of iodoacetate with thiol compounds became well established (Dickens, 1933; Quastel and Wheatley, 1932; Michaelis and Schubert, 1934; Hellström, 1931, 1932, 1934), it has often been assumed that the physiological action of this compound involved combination with an intracellular sulfhydryl compound.

The relative effects of iodoacetate and iodoacetamide on oxygen consumption, anaerobic glycolysis, respiratory quotient, and excitability of frog muscle and muscle preparations have been studied in this work. Iodoacetate was found to be a much more rapid inhibitor of muscle glycolysis than iodoacetamide, confirming Smythe's findings with yeast. The amide seemed superficially to be a more rapid inhibitor of the respiration thus duplicating the order for combination with thiol compounds, but the differences between the two compounds in their effects on respiration were dependent on the H ion concentration of the medium. No difference remained at pH 4.6. Both sets of results are interpreted as a negation of the suggested mechanism of inhibition by combination with sulfhydril groups. The experiments on respiration and glycolysis will be reported in this paper while the measurements of excitability and respiratory quotient which indicated further differences between the two compounds will be presented immediately following.

METHOD. Oxygen consumption and glycolysis were measured in a differential volumeter of the type described by Fenn (1927). The bottles

were of approximately 15 ml. capacity and the capillary averaged 3.5 $mm.^3/cm.$ Paired sartorius, semitendinosus, ileofibularis, semimembranosus, tibialis anticus longus, and in small frogs peroneus muscles were used averaging 200 mgm. to each bottle. The Ringer's solution contained NaCl, 0.65 per cent; KCl, 0.012 per cent; $CaCl_2$, 0.014 per cent; and phosphate buffer to yield pH 7.38 and 13.6 mgm. P per 100 ml. For glycolysis measurements the phosphate buffer was replaced by bicarbonate-CO_2 mixture.

The glycolysis was estimated in most experiments by displacement of CO_2 from 0.02 N bicarbonate in Ringer's solution equilibrated with 2 per cent CO_2 in nitrogen. The gas mixture was passed over copper at dull red heat. The final pH of this mixture was 7.7 at which point solutions of phosphocreatine and its hydrolytic products are isohydric. Meyerhof and Boyland (1931), Saslow (1936) and others have shown the estimation of CO_2 liberation from CO_2-bicarbonate systems to be a reliable index of lactic acid production at this pH. As a further check chemical estimations of the lactic acid produced were compared in preliminary experiments with the changes in CO_2 pressure under the present experimental conditions using Wendel's modification of the Friedmann, Cotonio and Shaffer method (Wendel, 1933) to determine the lactic acid.

Except where otherwise noted the iodo compounds were tipped onto the experimental material from a side arm in the experimental bottle after a suitable control period. Inhibitions were then calculated in *per cent* referred to the average control rate. In experiments with muscle mash and muscle extract where the control rate falls with time and in certain experiments with intact muscle, controls were run throughout the experiment in duplicate. In calculating the final concentration of iodo compound after tipping 80 per cent of the muscle wet weight was considered as water. The 1 per cent stock solutions of the iodo compounds were diluted with the appropriate Ringer's solution for each experiment, the iodoacetic acid being first neutralized to phenol red. Fresh stock solutions were prepared each week. The iodo compounds were prepared by Dr. David R. Goddard, University of Rochester, and carefully recrystallized.

The muscles were always dissected 3 to 20 hours before an experiment and equilibrated at 5°C. in oxygenated phosphate-Ringer's solution. The preparation of the mash and extract and other details will be described in connection with the specific results.

Results. *Glycolysis.* It was first shown by chemical estimation of the lactic acid that iodoacetamide did indeed inhibit the lactic acid formation of muscle. Five pairs of muscles were treated with 0.38×10^{-3} Molar iodoacetamide in Ringer-phosphate solution. One member of each pair was kept for two hours under anaerobic conditions in the experimental

solution, the other member for five hours before analysis. The muscles kept for two hours averaged 34.3 mgm. per cent lactic acid; those kept for five hours averaged 32.7 mgm. per cent lactic acid. Volumeter experiments shown in table 1 indicate that approximately two hours are required for inhibition of glycolysis at this concentration of the amide. Hence it can be concluded that very little lactic acid was produced in nitrogen between the second and fifth hours, and the action of iodoacetamide at this concentration is an inhibition of lactic acid formation.

The relative rates of inhibition of anaerobic glycolysis measured in the volumeter showed that iodoacetate is a much more rapid inhibitor of glycolysis than iodoacetamide. This is summarized in table 1 which

TABLE 1

Time for 80 per cent inhibition of anaerobic glycolysis

23° C.

IODOACETATE			IODOACETAMIDE			$\frac{T_{ac}}{T_{am}} = R$
Concentration	Number of experiments	T_{ac}	Concentration	Number of experiments	T_{am}	
$M \times 10^{-3}$		*minutes*	$M \times 10^{-3}$		*minutes*	
			1.6	1	32	
1.08	1	15	1.08	7	39	0.38
0.54	1	30	0.54	3	50	0.60
0.43	3	30	0.43	4	104	0.29
0.32	7	76	0.32	5	103	0.74
0.27	2	90	0.27	6	>240	<0.38
0.25		60*				
0.16	2	200				

* Data of Meyerhof and Boyland (1931).

presents average values for the time to produce 80 per cent inhibition at a series of concentrations in the range 0.16 to 1.6 $\times$ 10^{-3} Molar. The ratio $\frac{\text{Time for iodoacetate to produce a given inhibition}}{\text{Time for iodoacetamide to produce a given inhibition}}$ (henceforth referred to as R) is considerably below one.

It will be noted that the time for 80 per cent inhibition rather than complete inhibition is chosen. This was considered a more reliable criterion since in approximately half of the experiments the rate of CO_2 production increased again after approaching zero. This rate averaged only 10 to 15 per cent of the original control rate and never persisted long enough to account for more than 10 to 15 per cent of the total gas measured, although in extreme cases it might reach 100 to 200 per cent of the control rate for short intervals. This secondary CO_2 production is probably associated with combination of the iodo compounds with denatured protein and consequent liberation of HI (Mirsky, 1936a). In general,

iodoacetamide poisoning led to secondary CO_2 production more readily than iodoacetate poisoning. This might be expected since the reaction is with —SH groups.

A large difference between the two compounds in their rates of combination with denatured protein might be thought to alter seriously their effective concentrations for inhibition of glycolysis or respiration. Calculations based on Mirsky's data for the proteins of frog muscle (Mirsky, 1936b), *viz.*, 19 per cent of the muscle wet weight is protein and 1 per cent of this protein is cysteine, showed that if all the —SH groups reacted with one poison and none with the other and the entire combination took place during the time necessary to inhibit glycolysis a 24 per cent difference in effective concentrations would result. Actually only small amounts of denatured protein would be expected or only a small fraction of the total —SH would be available (Mirsky). Also both iodo compounds would be expected to react though at differing rates. Hence, no greater error than 10 per cent can be attributed to lowering of the effective concentration of one poison by its more rapid disappearance in combination with denatured protein. No greater error is introduced by assuming that the glycolytic enzyme is inactivated by denaturation of its protein, the —SH groups of this protein thus becoming available for combination with the iodoacetyl group.[1]

In cases where no secondary CO_2 production was observed the capillary index drop either remained stationary or indicated a slight absorption of gas. This latter might be due to inaccurate adjustment of the pH. In all cases the muscles were non-irritable and in typical rigor by the time secondary effects were measurable and it is concluded that a maximum error of 10 per cent is introduced by these factors.

The apparent threshold below which the poison is ineffective was lower for iodoacetate. This difference is probably more apparent than real because of the more rapid action of the acetate. The measurements were not usually continued longer than four hours.

A measure of the scatter to be expected in a series of experiments at any given concentration along with the values of R for 80 and 100 per cent inhibition is given in table 2. A similar degree of dispersion was obtained at the other concentrations recorded in table 1. The agreement among respirometers in a single experiment gave an average deviation of $\pm$ 7 per cent for the times to yield a given inhibition. Thus the observed differences between iodoacetate and iodoacetamide are concluded to be real.

Respiration. The threshold concentration for inhibition of the oxygen consumption by either compound is of course considerably higher than for inhibition of glycolysis (Lundsgaard, 1932). A series of experiments

[1] These calculations were stimulated by a speculative interpretation of the results by Dr. C. V. Smythe. (Private communication.)

similar to those recorded in table 1 is summarized in table 3 traversing the concentration range 0.11 to 16.2 $\times$ 10^{-3} Molar. Obviously the iodoacet-

TABLE 2

Inhibition of anaerobic glycolysis at 0.32 $\times$ 10^{-3} acetate or amide

23°C.

IODOACETATE		IODOACETAMIDE		R FOR 80 PER CENT	R FOR 100 PER CENT
Time for 80 per cent inhibition	Time for 100 per cent inhibition	Time for 80 per cent inhibition	Time for 100 per cent inhibition		
minutes	*minutes*	*minutes*	*minutes*		
75	75	90	105	0.83	0.71
90	105	100	110	0.90	0.95
60	75	105	105	0.57	0.71
55	75	110	110	0.50	0.68
70	80	110	110	0.64	0.73
70	80				
90	100				
Av....73 $\pm$ 10 (a.d.)	84 $\pm$ 10 (a.d.)	103 $\pm$ 7 (a.d.)	108 $\pm$ 4 (a.d.)	0.69 $\pm$ 0.14 (a.d.)	0.76 $\pm$ 0.08 (a.d.)

Muscles in bicarbonate-Ringer's at pH 7.7; 2 per cent CO_2 in nitrogen in the gas space. The time for 100 per cent inhibition does not include any secondary CO_2 production if such occurred. The ratio R is higher here than at higher concentrations (cf. table 1).

TABLE 3

Time for 50 per cent inhibition of respiration

23°C.

IODOACETATE			IODOACETAMIDE			R
Concentration	Number of experiments	T_{ac}	Concentration	Number of experiments	T_{am}	
$M \times 10^{-3}$		*minutes*	$M \times 10^{-3}$		*minutes*	
16.2	1	62	16.2	1	50	1.23
10.8	6	161	10.8	6	66	2.44
5.4	2	56	5.4	2	28	2.00
2.2	4	102	2.2	1	60	1.70
1.08 to 0.11	6	>240	1.08	4	113	>2.1
			0.81	2	108	
			0.54 to 0.11	15	>240	

amide is the more rapid respiratory inhibitor, exactly reversing the relations found for glycolysis. The value of R becomes greater than one under these experimental conditions.

The time for 50 per cent reduction rather than 80 per cent reduction is given. This was expedient since the inhibition of respiration is a relatively slow process. Identical relations were observed at higher concentrations of the iodo compounds for both 75 per cent and complete inhibition although the ratio of the times was not necessarily numerically identical (cf. table 4). Undue prolongation of the experimental period was necessary to obtain complete data at lower concentrations.

Table 4 presents a measure of the scatter obtained in the respiration experiments and a comparison of the times for 50 and 75 per cent inhibition. The dispersion is somewhat greater than in the data for glycolysis but the difference in activity of the two compounds seems real nevertheless.

TABLE 4

Inhibition of respiration at 10.8 × 10^{-3}M acetate and amide

23°C.

IODOACETATE		IODOACETAMIDE		R FOR 50 PER CENT	R FOR 75 PER CENT
Time for 50 per cent inhibition	Time for 75 per cent inhibition	Time for 50 per cent inhibition	Time for 75 per cent inhibition		
minutes	*minutes*	*minutes*	*minutes*		
97		62	145	1.56	
145		65	100	2.23	
200	260*	28	65	7.14†	4.0
115	205	58	85	1.98	2.41
230	370*	100	150	2.30	2.46
180	240*	80	90	2.25	2.67
Av..... 161 ± 43 (a.d.)	269 ± 51 (a.d.)	66 ± 27 (a.d.)	106 ± 28 (a.d.)	2.06 ± 0.24 (a.d.)	2.89 ± 0.56 (a.d.)

* Extrapolated value.

† Omitted from average.

The effect of pH on inhibition of the oxygen consumption. Figures 1 and 2 are inhibition curves taken from sets of paired muscles, one member of each pair at pH 7.2, the other member at pH 6.0 or 4.6 respectively. In both cases the difference between acetate and amide is greatly diminished. The ratio R approaches one. Other experiments not presented showed somewhat less marked diminution of the ratio at pH 6.0, *viz.*, *ca.* 50 per cent. The muscles were equilibrated in Ringer-phosphate solution of the same pH overnight. The possible significance of these results will be discussed below.

Muscle mash and muscle extract. To show that the differences recorded above are not limited to resting intact muscle the respiration and glycolysis of a muscle mash and the glycolysis of a muscle extract were studied.

A muscle mash prepared by mincing all of the hind-leg muscles in the cold and suspending in Ringer's solution exhibits rates of respiration and glycolysis two to four times as high as resting muscle. Experiments with

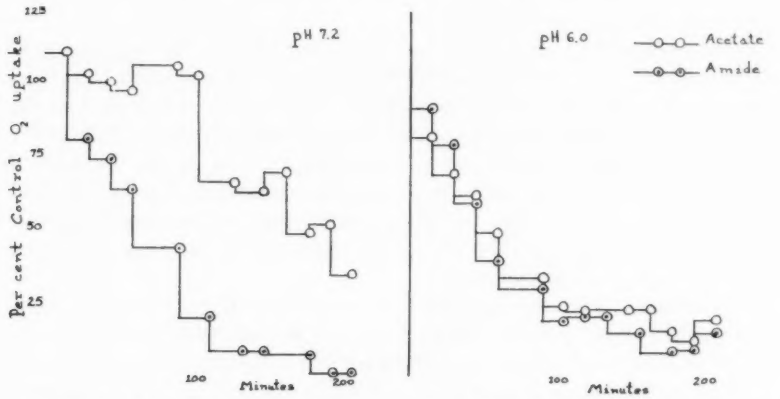

Fig. 1. Inhibition of respiration by 10.8×10^{-3} M iodoacetate and iodoacetamide at pH 7.2 and pH 6.0. One member of each muscle pair was at the lower pH, the other member at the higher pH. It will be noted that the comparison of acetate and amide involves unpaired muscles. Control experiments at constant pH show that this introduces no complications in interpreting these figures.

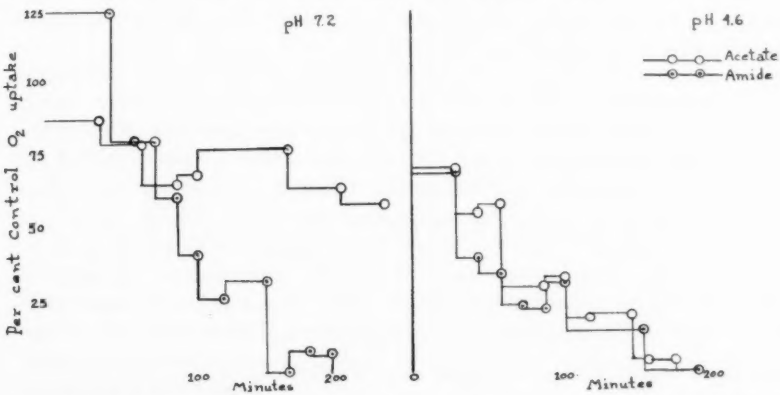

Fig. 2. Inhibition of respiration by iodoacetate and iodoacetamide at pH 7.2 and pH 4.6. Other details as in figure 1.

this preparation (with suitable controls for the fall in control rate with time) showed no marked change in the ratio R for either respiration or glycolysis when compared with intact muscle (table 5). The times re-

quired to produce a given effect were more irregular than in the case of intact muscle, possibly due to uncontrollable variations in the mashes. Three experiments indicated greater sensitivity of the respiration to both compounds. However, suitable controls were lacking and these experiments are omitted from table 5.

Only the glycolysis of the extract was measurable. The extracts were prepared on ice from finely minced muscle by water or isotonic KCl extraction for one hour and used immediately. "Kochsaft" was added at the beginning of an experimental period and either 0.2 per cent starch or 0.2 per cent glycogen solutions used as substrates. As shown in table 6 iodoacetate is by far the more rapid inhibitor of the glycolysis. In fact the difference between the two iodo compounds is considerably greater than in intact muscle, and the ratio R becomes smaller than those observed for intact muscle or muscle mash.

Discussion. The results obtained on muscle glycolysis agree with those obtained by Smythe (1936) for yeast fermentation. The physiological order of effectiveness of iodoacetate and iodoacetamide as measured by their relative rates of inhibition is exactly the reverse of the order of reaction with sulfhydryl groups *in vitro*. Unless unforeseen complicating factors exist it seems unlikely on the basis of the experiments reported here that inhibition of fermentation and glycolysis by the iodoacetyl group involves a reaction with sulfhydryl. No known sulfhydryl compound combines more rapidly with iodoacetate than with iodoacetamide. Lohmann (1933) has already advanced evidence that the inhibitory action of iodoacetate on the glycolysis of muscle extract is probably not due to combination with glutathione. A review of the possible effect of iodoacetate on the metabolism of plants has been published by Turner (1937). Dixon (1937) has shown that iodoacetate exerts a specific inhibitory effect on the alcohol dehydrogenase of yeast, and that the action in this case does not involve the coenzyme, but no specific effect was observed on various dehydrogenases of muscle at concentrations which inhibit glycolysis. Recently Schubert (1936) has found that amines of the pyridine group react with the iodoacetyl group at rates of the same order as thioglycolic acid, and that hexamethylenetetramine reacts with a velocity constant between those of thioglycolic acid and glutathione. Furthermore the acetate combines more rapidly than the amide. The ratio $\frac{\text{Time for iodoacetate}}{\text{Time for iodoacetamide}}$ is of the same general order of magnitude as that found in my glycolysis experiments. This latter work suggests a possible alternative mechanism for the inhibition since the coenzyme for muscle glycolysis and cozymase appear to be pyridine or nicotinic acid amide compounds (Warburg and Christian, 1936; Meyerhof and Ohlmeyer, 1936; Euler, Albers and Schlenk, 1935).

TABLE 5

Inhibition of respiration and glycolysis of muscle mash

23°C.

IODOACETATE			IODOACETAMIDE		R FOR 50 PER CENT	R FOR 80 PER CENT
Concentration	Time for 50 per cent inhibition	Time for 80 per cent inhibition	Time for 50 per cent inhibition	Time for 80 per cent inhibition		
Respiration						
$M \times 10^{-3}$	*minutes*	*minutes*	*minutes*	*minutes*		
5.4	50	120	35	60	1.43	2.0
1.35	90	150				
Glycolysis						
1.08	30	70	56	100	0.54	0.70
0.97	15	60	40	81	0.38	0.74
2.2	15	30	25	80	0.60	0.38
0.27			75	100		
0.11			100	220		

TABLE 6

Inhibition of glycolysis of muscle extract

23°C.

TIME AFTER TIPPING	IODOACETATE		IODOACETAMIDE		R FOR 80 PER CENT INHIBITION
	mm^3 CO_2/10 min.	Per cent of control rate	mm^3 CO_2/10 min.	Per cent of control rate	
Experiment A. 0.54×10^{-3}M					
First hour	0.13	5	0.90	39	
Second hour	0.10	6	0.41	24	
Third hour	0.06	5	0.30	26	0.071
Experiment B. 0.81×10^{-3}M					
First hour	0.51	80*	0.48	75	
Second hour	0.13	20	0.25	38	
Third hour	0.0	0	0.29	45	<0.33
Experiment C. 0.41×10^{-3}M					
First hour	0.17	17	1.11	89	
Second hour	0.16	14	0.79	63	
Third hour	0.17	17	0.63	50	<0.10

* This rate fell rapidly during the second 30 minutes and was nearly as low during this period as during the second hour.

Two milliliters of extract made 0.02 N with respect to bicarbonate, 0.2 ml. substrate solution, 0.25 ml. iodo compound in side-arm, 2 per cent CO_2 in nitrogen in gas space.

The experiments on respiration indicate superficially the order of effectiveness expected in a reaction with sulfhydryl compounds. However, the fact that the differences are eliminated at acid pH values suggests at least a partial interpretation on the basis of penetration. Since iodoacetamide is a neutral molecule of approximately the same mass as the ionized iodoacetate it would be expected to penetrate living tissues more readily than iodoacetate. In fact this compound was first employed as a physiological inhibitor for this reason (Goddard, 1935). At more acid pH values the ratio of salt to acid would be greatly decreased. If the undissociated acid penetrates more readily the iodoacetate should inhibit more rapidly at low pH than at high pH, as is actually found. Kohn (1935) obtained similar results for the poisoning of the photosynthetic mechanism in Chlorella and interpreted them in this manner.

The part played by penetration may be checked in another respect. If the amide penetrates more readily than the acetate at the pH of normal Ringer's solution, then the iodoacetate should inhibit glycolysis even more readily than it is observed to were it not for its slow penetration. In a cell free extract the value of R should decrease. This was true experimentally (table 6).

A specific effect of pH on the reactions concerned has of course not been completely eliminated. However the effect of pH on the interaction of the iodoacetyl radical and thiol compounds is the reverse of that found in these experiments (Smythe, 1936; Hellström, 1932).

Complication for a simple penetration explanation of the experiments on oxygen consumption is introduced by the data of Meyerhof and Boyland (1931) who found by chemical estimation of the iodine content of frog muscles as a function of time that "diffusion equilibrium" at 0.25×10^{-3} Molar iodoacetate in normal Ringer's was accomplished within 20 minutes. The time differences as well as the total times for inhibition recorded in table 3 are of a higher order of magnitude. A longer total time might be expected since considerable quantities of oxidizable intermediates may remain and the hypothesized destruction of the respiratory enzymes is probably a slow and indirect process. The former difficulty cannot be resolved without further experimental work, including direct determination of the relative penetration times of the two poisons. On the other hand there is no direct evidence from these experiments supporting the view that inhibition of the oxygen consumption involves interaction with sulfhydryl groups.

It was hoped that a kinetic analysis of the poisoning curves would cast light on the possible mechanisms concerned. However, too many factors are apparently involved to present sufficiently regular curves. The ratio $\frac{\text{Time for iodoacetate}}{\text{Time for iodoacetamide}}$ is not necessarily the same when different time criteria are chosen. This presumably indicates that the curves are of

different shape. Further differences in the action of these two compounds involving changes in the excitability and respiratory quotient will be presented in the following paper.

SUMMARY

1. Iodoacetate inhibits the anaerobic glycolysis of resting frog muscle, muscle mash or muscle extract much more readily than iodoacetamide.

2. This is exactly the reverse of the order of effectiveness known for these two compounds in their reaction with compounds containing the sulfhydryl group.

3. The amide inhibits respiration more quickly at physiological pH. However, both compounds are equally effective at more acid pH values, and the difference at physiological pH is accounted for in part by more rapid penetration of the amide.

4. Neither respiration nor glycolysis indicates on these criteria that the mechanism of inhibition by the iodoacetyl group involves combination with an intracellular sulfhydryl compound.

The writer is indebted to Dr. W. O. Fenn for his continued interest in the work, to Dr. D. R. Goddard for many stimulating conferences and the iodo compounds used, and to Dr. C. V. Smythe for several suggestions.

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THE RESPIRATORY QUOTIENT AND EXCITABILITY OF FROG MUSCLE TREATED WITH IODOACETATE AND IODOACETAMIDE

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In the previous paper (Stannard, 1938) certain quantitative differences in the action of iodoacetate and iodoacetamide on the respiration and glycolysis of frog muscle were noted. In addition qualitative differences in the action of these two compounds appeared in a study of the resting respiratory quotient and the excitability of muscles so poisoned. These results will be reported here.

The respiratory quotient of resting muscle whose glycolysis was prevented by 0.32×10^{-3} Molar iodoacetate was experimentally identical with that of resting unpoisoned muscle. On the other hand muscles similarly poisoned with iodoacetamide exhibited a rise in respiratory quotient to unity.

The excitability of iodoacetate-poisoned muscle as measured by changes in the rheobase and shape of the strength-duration curve seems first to rise (the muscles become more excitable) and then gradually fall as contracture ensues. In iodoacetamide no change appeared until the initiation of contracture when the results duplicated those with iodoacetate. The interpretation of these changes is as difficult as a suitable chemical interpretation of either the respiratory quotient or excitability in normal frog muscle. Therefore, the following results are presented essentially as a recording of experimental data.

The respiratory quotient. The experiments were carried out in collaboration with Dr. George Saslow whose series on the R.Q. of caffeinized muscle (Saslow, 1937) they paralleled. The same method and same lot of frogs were employed and often controls served for both series of experiments.

The usual thin hind-limb muscles of two or more frogs were equilibrated 3 to 18 hours in oxygenated phosphate-Ringer's solution (pH 7.38) at 5°C. Four respirometers were necessary for an experiment. Approximately 2 grams of muscle were utilized for the R.Q. determination, one member of each pair for preformed CO_2 (respirometer 1) and the other for preformed plus respiratory CO_2 (respirometer 2). The remaining pairs were divided

for determination of the oxygen consumption without iodo compound (respirometer 3) and anaerobic glycolysis in the presence of the iodo compound (respirometer 4). All muscles were placed in the respirometer bottles as nearly simultaneously as possible. The poison concentrations were adjusted so that the final concentration, assuming 80 per cent of the muscle wet weight to be water, was 0.32×10^{-3} Molar. At this concentration of either poison, glycolysis can be completely inhibited with but slight effect on the oxygen consumption (Lundsgaard, 1930; Meyerhof and Boyland, 1931; Saslow, 1936; Stannard, 1938).

Measurements of the glycolysis were started immediately, the other three respirometers being shaken and oxygenated meanwhile. As soon as the glycolysis had ceased the taps were turned in the other respirometers. After two ten-minute readings to check for divergences in the O_2 uptake among the volumeters 2.5 N HCl was tipped in one bottle, first into the barium hydrate, then onto the muscles to determine the preformed CO_2. After an experimental period gauged so that the fraction $\frac{\text{Preformed } CO_2}{\text{Total } CO_2}$ was less than 25 per cent, the contents of another respirometer were similarly acidified to determine the preformed plus respiratory CO_2. The last respirometer contained no iodo compound, serving simply as a respiration control. The general precautions outlined by Dickens and Šimer (1930) and by Fenn (1932) were taken into account. For other details see the previous paper (Stannard, 1938) and Saslow (1937).

The respiratory quotient measured was thus that of muscles whose glycolysis was known to be inhibited, and the measurement started after complete glycolysis inhibition. The fact that the muscles for the glycolysis measurement were in bicarbonate-Ringer's and anaerobic while the others were in phosphate-Ringer's and aerobic does not alter the rate of inhibition (Saslow, 1937). Iodoacetate required 75 to 100 minutes (average 95 min.) while the amide required 105 to 110 minutes (average 107 min.) to effect this inhibition.

Table 1 presents a summary of the results obtained. Comparison of the average R.Q. in iodoacetate with the values for normal muscle recorded by Fenn (1932) and Saslow (1937), *viz.*, 0.87 and 0.89 respectively, show no essential change in the presence of this compound. Gemmill's most recent value (1934) for resting muscle by the manometric method was 0.80. Saslow's value may be considered a complete control for these experiments since it was obtained by the same method, at the same time, on the same lot of frogs.

Meyerhof and Boyland (1931) found the R.Q. of iodoacetate-poisoned muscle to be 0.8 to 0.7, thus indicating a possible increase in the proportion of fat oxidized. My experiments would lead to the conclusion that no essential change had occurred in the resting oxidative metabolism in spite

of complete inhibition of glycolysis. The only obvious differences between these experiments and those of Meyerhof and Boyland are: (1) In the latter case the poison was not in contact with the muscles during the actual experimental period. (2) The rates of respiration in Meyerhof and Boyland's poisoned muscles were considerably lower than in these experiments. (3) The amounts of muscle used in our experiments were larger.

TABLE 1

Respiratory quotient in iodoacetate and iodoacetamide, 0.32×10^{-3} Molar

DATE	WEIGHT OF MUSCLES	DURATION OF EXPERIMENT	AVERAGE O_2 USED BY POISONED MUSCLE	AVERAGE O_2 USED BY UNPOISONED MUSCLE	PREFORMED CO_2*	TOTAL CO_2	R.Q.
Iodoacetate							
	grams	*hours*	*mm^3/g/hr.*	*mm^3/g/hr.*	*mm^3/gm.*	*mm^3/gm.*	
January 18	0.620	5	72		61.2	352.3	0.81
January 19	1.126	4.3	42.3	41.3	25.5	187.0	0.89
January 20	1.091	4.5	45.5	51	22.7	217.2	0.95
January 21	0.972	4	51.2	59	22.4	215.0	0.94
January 22	0.879	8	24.2	44	47.3	225.3	0.92
January 23	1.018	4	39.8	25	24.1	175.4	0.95
January 25	1.201	4	34.2	30	36.4	160.8	0.91
Average	0.987	4.9	44.0	42.0	34.2	219.0	0.91
Iodoacetamide							
January 26	1.016	4	37.2	25	23.8	183.0	1.07
January 27	1.213	4.17	36.9	25	37.2	192.4	1.01
January 29	0.990	6	27	27	30.5	200.6	1.05
February 1	1.332	5	34	22	43.6	216.9	1.02
February 5	1.223	4	44.1	24	27.8	212.9	1.05
April 11	1.463	4.67	29		50	182.8	0.98
April 11	1.442	3.2	30		50	134.5	0.88
Average	1.240	4.5	34.0	24.6	34.7	189.0	1.008

* This value includes the respiratory CO_2 absorbed by the barium hydrate while waiting for glycolysis to be inhibited.

(4) The experimental temperatures differed, being 15°C. in Meyerhof and Boyland's work, and 23°C. in this work. (5) The muscles were equilibrated several hours at 5°C. before the determinations in these experiments, i.e., the muscles were used a longer time after dissection. Whether or not any of these factors account for the observed discrepancies is not clear. Since iodoacetate poisoning is irreversible, item 1 might be eliminated. It will be recalled that Fenn (1932) was inclined to attribute the

difference between his resting R.Q. values and those of Meyerhof on the basis of the longer time involved in the Rochester experiments.

The R.Q. in iodoacetamide-poisoned muscles is distinctly higher than that of resting or iodoacetate muscle. The average value of approximate unity indicates almost complete carbohydrate oxidation with a presumptive elimination of fat and whatever small amount of protein oxidation may occur in the normal resting muscle. If the low value of 0.88 be omitted as aberrant the average R.Q. is even slightly above unity (1.03).

No factor or experimental condition obvious to the writer can account for this difference between iodoacetate- and iodoacetamide-poisoned muscles. An experiment on unpoisoned muscle on February 2 in the same apparatus gave an R.Q. of 0.93. Iodoacetate muscle can easily oxidize added lactate (Meyerhof and Boyland, 1931). I have found the same to be true of iodoacetamide muscle (unpublished experiments). Therefore we might expect any excess lactate to be oxidized and the increased oxidation reflected in the rate of oxygen consumption. Since the amide requires a longer time to inhibit glycolysis (Stannard, 1938), more lactate might be expected to accumulate in this case. But the average rates of oxygen consumption of the poisoned muscles agree well with the unpoisoned controls (table 1). There is no obvious difference in the rates which would be expected if one set of muscles were oxidizing increased amounts of lactate. Furthermore, calculations of the amount of lactate needed to account for the total oxygen consumption of iodoacetamide muscle requires more than the expected amounts in many experiments although the average requirement, 25 mgm. per cent, might account for the oxygen consumption if the entire lactate content were oxidized. In addition, the data of Hegnauer, Fenn and Cobb (1934) negate the rôle of lactate as pacemaker for the O_2 consumption.

Hydrolysis or oxidation of the iodo compounds themselves cannot account for the differences. With the quantities employed complete oxidation or hydrolysis of either compound would result in less than 1 $mm.^3$ additional CO_2. Similarly, differences in the lability of the compounds or retention of CO_2 are without significance. The final pH values were identical to the glass electrode.

An attempt to account for the rise in R.Q. in iodoacetamide-poisoned muscle by chemical analysis has not been undertaken, since a complete balance sheet would be required, and it is not yet clear exactly what substrate is oxidized by normal resting muscle. The possible selective inhibition of fat oxidation should be investigated and the possibility that protein is oxidized with ammonia as end-product should be checked. This latter accounted for the high R.Q. of fluoride-posioned chick embryos in the experiments of Needham (1932).

There is no reason *ad hoc* to expect the amide linkage to alter the essen-

tial properties of the iodoacetyl group on the glycogenolytic system. But effects on other less well-understood non-carbohydrate systems may be found responsible for the observed changes. The data obtained give no indication that frog muscle whose lactic acid production is completely inhibited by either iodo compound is unable to oxidize carbohydrate at the normal resting rate. In this sense, these results fall in line with the accumulating evidence from many sources (cf. Needham, 1937) that the oxidative substrate in muscle is not necessarily lactate.[1]

Excitability. The α excitability was measured by means of direct current stimuli using the expression derived by Blair (1935) in the form,

$$\log \frac{V}{V - R} = kt + C$$

where V is the applied voltage, R the rheobase, t the duration of the stimulus in milliseconds, and k and C are constants. The experiments were carried out in July, 1937, in collaboration with Dr. H. A. Blair, to whom the writer is greatly indebted. The apparatus which is described by Blair (1937) determines the strength-duration curve of the muscle substance (α curve) rather than nerve.

Sartorius muscles were equilibrated at 5°C. overnight. Control measurements were first carried out in phosphate-Ringer's solution. Ringer's solution containing the iodo compound in the desired concentration was then substituted and the measurements continued as quickly as possible. Usually the muscles were finally returned to ordinary Ringer's solution but very little reversal of the effects occurred. Essentially the changes ran the same course, although at different rates, whether the poison was in actual contact for 15 minutes or 2 hours.

Considerable difficulty was experienced in securing reproducible rheobases or strength-duration curves after the poisoning had begun. Less than half of the experiments were usable. For this reason a more detailed consideration was thought inadvisable. Yet sufficient data were obtained to indicate a real difference between the two iodo compounds which seemed worth recording in conjunction with the other differences already noted.

Figures 1 and 2 show the changes in rheobase and k as a function of time after the application of the iodo compound for iodoacetate and iodoacetamide respectively. The constant k, the usual measure of excitability, was obtained from the slope of the curve relating $\log \frac{V}{V-R}$ and the duration of the stimulus in milliseconds (Blair, 1932).

The rheobase in iodoacetate invariably falls as a primary stage. Second-

[1] Recently Shorr, Barker, and Malam (Science, **87**: 168, 1938) have shown that mammalian tissues treated with sufficient iodoacetate to completely inhibit glycolysis yet allow satisfactory maintenance of respiration exhibited no change in respiratory quotient. Lactate analyses showed that this substance could not be acting as substrate for the oxidation, and the authors conclude that the ability to oxidize glucose is not dependent upon lactate formation.

arily the muscle becomes gradually inexcitable and the typical iodoacetate rigor follows. In iodoacetamide no primary depression of the rheobase occurs. The primary phase is instead a slow rise in rheobase, followed, less quickly than with iodoacetate, by a more rapid rise and the onset of rigor. The curves are shifted to the left by increasing concentrations indicating that the changes in excitability are related to the presence of the iodo compound.

The curves for k as a function of time seem to vary in the same direction as the rheobase. The number of satisfactory observations of k was greatly limited by the instability of the muscles. No k was plotted unless the

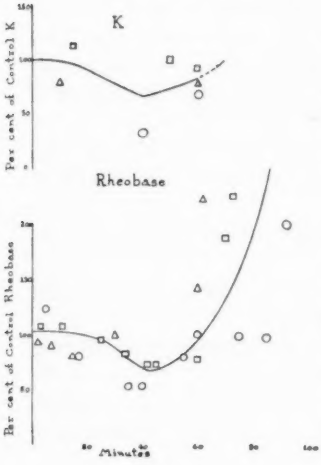

Fig. 1

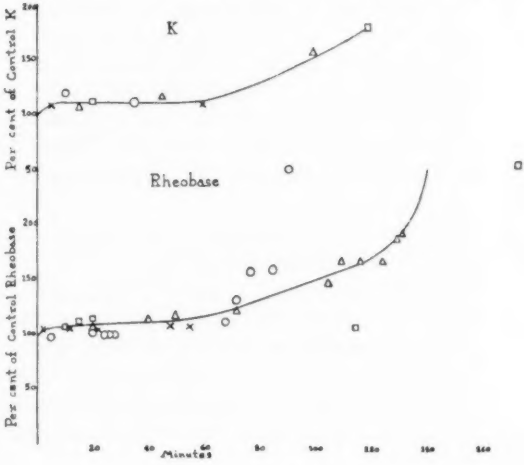

Fig. 2

Fig. 1. Change in rheobase and k in iodoacetate. Zero time corresponds to the moment of contact with the iodo compound. △ 0.38×10^{-3} M; ⊡ 0.27×10^{-3} M; ⊙ 0.05×10^{-3} M.

Fig. 2. Change in rheobase and k in iodoacetamide. Zero time corresponds to the moment of contact with the iodo compound. ⊡, ×, and △ 0.27×10^{-3} M; ⊙ 5.0×10^{-3} M.

strength-duration curve was reproducible. Usually the changes were occurring too rapidly to permit the satisfaction of this criterion. Yet it can be concluded that during the primary stages k and the rheobase are not entirely dissimilar in their variations with time. This observation is of interest since, if the effect of the iodo compound is on the kinetics only of the excitatory process rather than on the threshold, which presumably depends on the state of the membrane, k and the rheobase should vary with each other. Thus a tendency of k and the rheobase to vary together in the early stages would indicate, perhaps, that some factor in the interior of the cell rather than the membrane is being affected. The final abrupt

rise of the rheobase may, of course, be due to a rapid rise of the threshold. No measurements of k were possible during this phase.

It is regrettable that more quantitative measures could not be applied, but the instability of the poisoned muscles precluded any further analysis. On the other hand, the data are sufficiently reliable to support the conclusion that these two iodo compounds have different gross effects on excitability. Possibly the acetate has two effects whose resultant we measure, while the amide has only one, a gradual decrease in excitability as the phosphocreatine stores are depleted.

SUMMARY

1. The respiratory quotient of resting frog muscle whose glycolysis has been completely inhibited by 0.32×10^{-3} Molar iodoacetate was 0.91. This value is sufficiently like those obtained in this laboratory for unpoisoned muscle to be considered identical. Thus iodoacetate inhibition of glycolysis does not seem to alter the resting oxidative metabolism.

2. The R.Q. of muscle similarly poisoned with iodoacetamide is unity. Iodoacetamide inhibition of glycolysis seems to alter the resting oxidative metabolism.

3. The rates of respiration were not appreciably changed by either poison during the experimental period.

4. Neither compound appears to interfere with the ability of the resting muscle to oxidize carbohydrate at the normal rate.

5. Iodoacetate first increases then decreases the α excitability of frog muscle. The decrease is associated with the onset of rigor.

6. The phase of increased excitability does not appear in iodoacetamide, while the second phase of decreasing excitability is similar to that seen in iodoacetate.

Dr. George Saslow and Dr. H. A. Blair collaborated directly in obtaining the experimental results. I am greatly indebted to them and to Dr. W. O. Fenn for his many suggestions.

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THE REDUCTION OF EXPERIMENTAL POLYCYTHEMIAS BY LIVER ADMINISTRATION

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In 1935, Marshall (1) reported that the injection of liver extract into the thigh muscles of polycythemic rats caused a temporary but distinct fall in the erythrocyte number. These rats were made polycythemic by the daily feeding of a milk-mineral diet containing cobaltous chloride. The feeding of whole liver, however, caused an increase in the already high erythrocyte counts of these rats.

Since it might be argued that liver could possibly counteract cobalt chemically and thus depress its stimulating effect on hematopoietic activity, we decided to re-investigate the problem of anti-anemic treatment of experimental polycythemias using cobalt, but also another method of producing polycythemia. A more natural means of inducing polycythemia is to be found in physical exercise. It has been shown by Davis and Brewer (2) that daily treadmill running or swimming for four to five weeks will produce in dogs a significant chronic increase of the erythrocyte number as well as the blood volume.

We therefore decided to use these two methods to increase the red corpuscle counts in two separate series of dogs: 1, daily exercise and 2, cobalt feeding. That the oral administration of cobalt will increase the erythrocyte number of dog's blood has been shown by Mascherpa (3) and Davis (4).

PROCEDURE. Eleven experimental dogs were used in these studies. After stabilization on a constant adequate diet of purina dog food which was continued throughout the experiments, four dogs were given daily treadmill running exercise (one-half hour duration at five miles per hour; treadmill inclined at 25 per cent grade); while the others were given 8 mgm. of cobaltous chloride per kilogram of body weight daily in solution 1:1000 by stomach tube.

Erythrocyte counts, hemoglobin percentage (Sahli), and reticulocyte counts (in three dogs only; by the technique of Wakerlin (5)) were made at frequent intervals, and leukocyte counts were made less frequently, throughout the pre-cobalt and pre-exercise control periods and the experimental periods.

Blood was drawn from the external saphenous vein while the dogs were lying quietly, blindfolded, upon a table at least eighteen hours after previous exercise, feeding, or treatment of any kind.

Results. The four dogs whose erythrocyte numbers appear in figure 1 had received daily exercise on a treadmill for at least six weeks prior to the start of liver feeding, and the exercise was continued as a hematopoietic stimulating measure throughout the experiment. Figure 1 shows the effect of feeding 75 grams of whole beef liver daily to each dog over periods ranging from four to thirteen days. It will be seen that in each dog the red cell count was reduced within two days after the commencement of liver feeding, that these reductions ranged from 15 to 24 per cent and

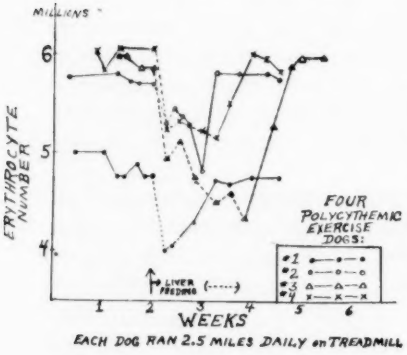

Fig. 1

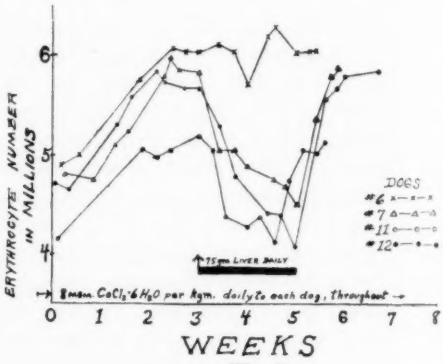

Fig. 2

Fig. 1. The reduction of erythrocyte numbers in four polycythemic exercise dogs by the feeding of 75 grams of liver daily. Broken line denotes period of liver feeding for each dog.

Fig. 2. The effect of oral liver administration upon the *erythrocyte numbers* of four dogs in which polycythemia is induced by cobalt chloride.

persisted throughout the periods of liver feeding. Upon cessation of liver administration, the erythrocyte numbers returned gradually (within about five days) to their previous higher levels. The hemoglobin percentages (not shown) followed the general trend of the erythrocyte numbers. Total leukocyte counts remained quite constant throughout the experiments.

Seven dogs, six of which are represented in figures 2, 3, and 4, were fed 8 mgm. of cobaltous chloride per kilogram of body weight daily. The cobalt caused increases, varying from 13 to 25 per cent, in the erythrocyte numbers of all seven dogs within two weeks. In spite of continued cobalt feeding, the oral administration of 75 grams of beef or hog liver daily caused a reduction of the erythrocyte number in six of the seven dogs to

or below the pre-cobalt normal values. These decreases in red cell count occurred within two to four days and persisted throughout the periods of liver ingestion, i.e., for eleven to twenty-eight days. Upon cessation of liver feeding the erythrocyte numbers returned to their previous cobalt-induced polycythemic values. The total leukocyte counts (not shown) were *not* reduced by the liver feeding.

The percentage of reticulocytes in the circulating blood was studied in three dogs, two of which are represented in figures 3 and 4. Cobalt administration caused an increase of at least 200 per cent over the observed normal reticulocyte percentages in all three of the dogs studied. When liver was fed in addition, the reticulocyte percentages decreased to or

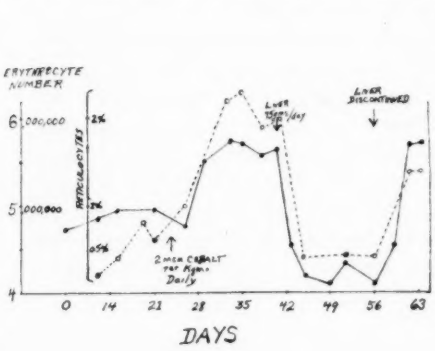

Fig. 3

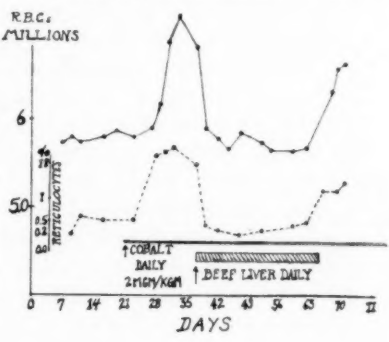

Fig. 4

Fig. 3. The attainment of polycythemia in dog number 10 by daily cobalt administration and its reduction by liver feeding. Solid line—erythrocyte number; broken line—reticulocyte percentage.

Fig. 4. The depression of cobalt polycythemia in dog number 9 for four weeks by the daily oral administration of beef liver. Solid line—erythrocyte number; broken line—percentage of reticulocytes.

below normal in close correspondence with the erythrocyte numbers. They remained low during the liver feeding periods and increased upon discontinuation of the liver.

Liver extract was injected intramuscularly into two cobalt-fed dogs and two exercise dogs (not shown in figures). Each dog received 0.5 cc. (1 cc. containing material from 33 grams of fresh liver) daily for six days. After four days of extract injections, all of the four dogs showed reductions in erythrocyte number, varying from 12 per cent to 20 per cent. Since we used an extract[1] which contained 0.5 per cent of phenol, it was necessary to rule out the possibility that the phenol was responsible for the effects of the extract. The injection of 1 cc. of 0.5 per cent phenol solution daily

[1] Valentine's Parenteral Liver Extract.

into the gluteus muscles of two dogs for six days, however, caused no fall of erythrocyte numbers.

Stomach extract (ventriculin) was fed to three polycythemic exercise dogs in daily doses of 5 grams, but *failed* to alter significantly the red cell counts although it was fed over a period of seven days.

Liver extract was administered by stomach tube to the same three dogs after a lapse of time. One cubic centimeter of this extract represented about 9 grams of fresh liver. Although 22 cc. were given to each dog daily for six days, no significant change in the erythrocyte numbers was observed during this time.

As a control, beef muscle was fed to three dogs over a period of six days without producing any real alteration in red cell counts.

Discussion. The fact that whole, raw, beef or hog liver feeding reduced the red corpuscle counts but not the leukocyte counts in four out of four exercised dogs and in six out of seven cobalt-fed dogs seems to furnish conclusive evidence that some constituent of the liver is effective in lowering a high erythrocyte number. We cannot explain the one exception in our experiments (dog 6, fig. 2) but can only observe that this dog was a female apparently in good health, but was a highly nervous animal.

Since the reticulocyte percentages observed in three polycythemic cobalt-fed dogs (see figs. 3 and 4) were markedly reduced by liver feeding, it is concluded that liver acts on such animals by *depressing* the hematopoietic activity of the bone marrow.

That the active constituent is probably not identical with any anti-anemic principles seems to be indicated by the fact that ventriculin, as well as oral anti-anemic liver extract administration, *failed* to alter significantly the erythrocyte numbers of three polycythemic exercise dogs.

The most conspicuous difference between the "successful" parenteral extract and the oral liver extract is that the latter is held at a high temperature for some time during the process of preparation while the former is not. Our dogs were fed raw liver except in the cases of two dogs which refused to eat it. These animals were given liver which had been boiled in a small amount of water for less than three minutes.

Concomitant changes in the dogs were ruled out, since we performed the different experimental procedures on various dogs at different times and seasons of the year, ranging from February through November.

Eight of our dogs were obtained and studied in Virginia; the others were natives of Alabama. Hence a small geographical distribution of animals is represented in our experiments.

Our results can be interpreted by assuming that a liver hormone exists which has the function of depressing the hematopoietic activity of the bone marrow.

CONCLUSIONS

1. The attainment of an increased erythrocyte number in dogs due to the daily ingestion of $CoCl_2$ is accompanied by an increase of over 200 per cent in the reticulocyte count and is therefore due to stimulation of the red bone marrow.

2. In six out of seven cobalt-fed, polycythemic dogs the oral administration of whole beef or hog liver (75 grams per dog daily) reduced the erythrocyte numbers to or below normal within two to four days. These reduced erythrocyte numbers were maintained throughout the duration of liver feeding (up to four weeks) in spite of continued daily cobalt administration. Reticulocyte counts taken on three dogs were markedly diminished during liver feeding. Leukocyte counts remained fairly constant. Upon cessation of liver administration, the erythrocyte numbers returned to their polycythemic levels within four days.

3. In four dogs in which polycythemia had been induced by daily treadmill running exercise, the oral administration of whole hog or beef liver caused a fall in the erythrocyte numbers and hemoglobin percentages to or below the normal levels of the pre-exercise period in spite of continued daily exercise. The reduced erythrocyte numbers persisted throughout the liver feeding (up to two weeks) and returned to polycythemic levels (about 20 per cent higher) promptly upon discontinuation of liver feeding.

4. The daily intra-muscular injection of 0.5 cc. of liver extract (1 cc. equal to material from 33 grams whole liver) into two polycythemic cobalt-fed dogs and two polycythemic (exercise) dogs caused significant (12 per cent to 20 per cent) decreases in red cell counts, which returned to previous levels soon after discontinuation of liver extract injections.

5. The daily oral administration of 5 grams stomach extract (ventriculin) and of anti-anemic liver extract heated during preparation did *not* reduce significantly the high erythrocyte counts of three dogs which received daily treadmill exercise.

6. These results are interpreted by the postulation of a liver hormone, quite distinct from anti-anemic principles, which has the function of depressing the hematopoietic activity of the red bone marrow.

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THE PROBABLE RÔLE OF CALCIUM AND INDIGO IN CELLULAR RESPIRATION

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In the preliminary paper (1) it was shown that respiration of excised tissues was not as great in citrate-Ringer's[1] (2) solution as in indigo-Ringer's[2] or cerebrospinal fluid. It seemed desirable to investigate the reasons for this difference.

Since the object was not to study a wholly artificial system where the observations might not apply in vivo, it seemed further desirable, as has been pointed out by Meldrum (3), to perform the experiments under conditions approaching as nearly as possible those existing in the animal body. In the use of excised tissues, however, it must be recognized that one is dealing with cells dying as a result of mere excision, and that further violence, such as cutting, slicing, mincing, or handling, should be reduced to a minimum if the life processes of the cell are to predominate over the processes attendant upon death and disintegration, and if, consequently, respiratory measurements are to have any biological significance. The tissues used consisted of liver of mouse and liver, kidney, and heart of rat, which needed slicing as well as excision, and of abdominal muscle of mouse, which needed only excision and which, having suffered the least injury, might serve somewhat as a check on the other tissues. Their respiration was measured by the Warburg differential method (4), because it permitted the use of liquid and gas media most nearly approaching those existing in the animal body. Both the excision and the preparation for the subsequent measurement were carried out as speedily as possible, in a room whose temperature was kept at 37 $\pm 0.5°$, in order to facilitate equilibrium between the tissue and the gas and liquid phases in which it respired. Even under the most favorable conditions possible, it could scarcely be expected that tissues would respire exactly the same after excision, when dying, as before, when living. The results, therefore, will

[1] Modified Ringer's solution: NaCl, 114 mM/L; $NaHCO_3$, 25 mM/L; KCl, 6 mM/L; $CaCl_2$, 1.4 mM/L; Na_3 Citrate, 0.7 mM/L; Na_2HPO_4—NaH_2PO_4, 1.0 mM/L; Dextrose, 0.2 per cent. (Ca^{++} = 1.0 mM/L, PH = 7.4.)

[2] Same as 1, but with Na_3 Citrate replaced by sodium indigo disulfonate, 0.1 mM/L.

have little significance beyond giving a clue whose value must be determined by other less objectionable methods.

Three series of experiments were performed in order to compare the effects of various liquid media on tissue respiration. In the first series, the liquid medium was human cerebrospinal fluid, from patients with various disorders of the central nervous system; in the second, it was Ringer's solution modified by various concentrations of calcium, phosphate, magnesium, and citrate; in the third, it was a modified Ringer's solution containing no calcium. The effect of adding methylene blue and sodium indigodisulfonate or tetrasulfonate to each of these media was also determined. The average oxygen consumption values obtained for each group of experiments are shown in table 1.

Cerebrospinal fluid was found to be the most satisfactory medium, inasmuch as in it the tissues respired at a maximum, with a minimum of glycolysis (not shown in the table). In the light of the results obtained with indigosulfonate-Ringer's solution as shown below, it would seem probable that cerebrospinal fluid might have exerted its favorable effect on respiration through the medium of its calcium ion supersaturation, a condition generally recognized (5, 6) as existing in most of the body fluids. The cerebrospinal fluid results have been placed first in order to furnish a point of departure for making comparisons with those of subsequent series. Attention is drawn to the fact that the addition of calcium to cerebrospinal fluid did not increase the respiration, whereas dilution lessened it somewhat, and that in no case did the addition of indigosulfonate exert an appreciable effect, whereas in every case methylene blue caused an appreciable increase.

In the second and third series of experiments, the standard liquid medium consisted of four constant constituents, namely, NaCl, KCl, $NaHCO_3$, and dextrose, and of four variable constituents, namely, $CaCl_2$, $MgCl_2$, $NaH_2PO_4-Na_2HPO_4$ and Na_3 citrate. It is noteworthy that the seven variations of series II, and the two of series III, caused so little difference by themselves in the respiration of the various tissues. At first sight, these results do not seem plausible; but they are capable of explanation on the basis that these changes have tended, on the one hand, to decrease respiration by interfering with the normal environment of the cell, and, on the other hand, to increase respiration by simultaneously exciting the cell to overcome the handicap imposed upon it. The effect that predominated would determine whether there would be increased or decreased respiration in a particular case, but in a number of cases the increases and decreases would probably balance, making the average effect nil.

Although there was little difference in respiration in the various media of the second and third series by themselves, there was considerable

TABLE 1

The oxygen consumption of excised rat and mouse tissues in cerebrospinal fluid (C.S.F.) and Ringer's solution as affected by variations in the concentration of the constituents and by the addition of methylene blue (M.B.) and indigosulfonate (I.S.)†

EXPERIMENTS		TISSUE	MEDIUM					AVERAGE OXYGEN CONSUMPTION IN		
Series of	Number of		Constants	Variables				Medium only	Medium + M.B., 0.1mM/L.	Medium + I.S., 0.1mM/L.
								cu.mm./mgm./hr.	Per cent change	Per cent change
I 1a	23	Rat liver	C.S.F.	None				16.0	+40	+3
I 1b	22	Rat kidney	C.S.F.	None				21.2	+44	−3
I 1c	20	Mouse liver	C.S.F.	None				16.8	+38	+1
I 1d	21	Mouse abd. M.	C.S.F.	None				6.8	+22	+2
I 2a	23	Rat kidney	C.S.F.	50 per cent dilution with Ringer's sans Ca				18.0	+40	−3
I 2b	22	Mouse abd. M.	C.S.F.	50 per cent dilution with Ringer's sans Ca				5.4	+23	+2
I 3	22	Rat liver	C.S.F.	Addition of Ca, 1.0 mM/L				15.8	+37	+3
				Ca mM/L.	PO_4 mM/L	Mg mM/L	Cit. mM/L			
II 1a	31	Rat liver	Ringer's*	1.0	1.0	0.0	0.0	11.4	+40	+45
II 1b	23	Mouse liver	Ringer's	1.0	1.0	0.0	0.0	12.4	+42	+40
II 1c	36	Mouse abd. M.	Ringer's	1.0	1.0	0.0	0.0	5.4	+35	+30
II 2a	25	Rat liver	Ringer's	1.0	0.0	0.0	0.0	11.3	+44	+20
II 2b	21	Rat kidney	Ringer's	1.0	0.0	0.0	0.0	15.6	+39	+20
II 2c	20	Mouse abd. M.	Ringer's	1.0	0.0	0.0	0.0	5.0	+45	+15
II 3	24	Rat liver	Ringer's	2.0	1.0	0.0	0.0	12.0	+45	+40
II 4	24	Rat liver	Ringer's	2.0	2.0	0.0	0.0	12.5	+49	+32
II 5a	23	Rat liver	Ringer's	0.5	0.0	0.0	0.0	10.1	+40	+14
II 5b	18	Rat heart	Ringer's	0.5	0.0	0.0	0.0	5.5	+36	+10
II 5c	19	Mouse abd. M.	Ringer's	0.5	0.0	0.0	0.0	4.8	+35	+10
II 6a	22	Rat liver	Ringer's	1.0	1.0	0.5	0.0	12.5	+50	+40
II 6b	22	Rat kidney	Ringer's	1.0	1.0	0.5	0.0	16.2	+44	+35
II 6c	23	Mouse abd. M.	Ringer's	1.0	1.0	0.5	0.0	5.6	+48	+40
II 7a	23	Rat liver	Ringer's	1.4	1.0	0.0	0.7	11.6		
II 7b	45	Rat heart	Ringer's	1.4	1.0	0.0	0.7	5.7		
II 7c	25	Mouse liver	Ringer's	1.4	1.0	0.0	0.7	13.0		
II 7d	34	Mouse abd. M.	Ringer's	1.4	1.0	0.0	0.7	5.6		
III 1a	18	Rat liver	Ringer's	0.0	1.0	0.0	0.0	11.0	+30	−2
III 1b	17	Rat kidney	Ringer's	0.0	1.0	0.0	0.0	15.0	+36	−2
III 1c	20	Mouse abd. M.	Ringer's	0.0	1.0	0.0	0.0	4.7	+35	−1
III 2a	30	Rat liver	Ringer's	0.0	0.0	0.0	0.0	10.9	+35	−2
III 2b	25	Rat kidney	Ringer's	0.0	0.0	0.0	0.0	15.2	+46	−1
III 2c	46	Rat heart	Ringer's	0.0	0.0	0.0	0.0	5.2	+30	+2
III 2d	36	Mouse abd. M.	Ringer's	0.0	0.0	0.0	0.0	4.8	+45	+1

* Constant constituents: NaCl, 114 mM/L; $NaHCO_3$, 25 mM/L; KCl, 6 mM/L; dextrose 0.2 per cent.

† Sodium indigodisulfonate or sodium indigotetrasulfonate of which the latter generally gave the highest values.

difference with the addition of indigosulfonate or methylene blue. When indigodisulfonate or tetrasulfonate was added, there was almost no change if calcium was not present as in series III, but about forty per cent increase

if calcium and phosphate were present, as in series II. When methylene blue was added, there was about forty-five per cent increase whether calcium was present or absent. Obviously, methylene blue and indigosulfonate differed in their mode of action.

The increased respiration caused by methylene blue has been generally attributed to its catalytic effect by virtue of its action as a reversible oxidation-reduction dye (7, 8). When added to the liquid medium in these experiments, it was reduced by the tissue cells toward the leuco form, but it never became completely reduced owing to its conversion to the blue form by the oxygen present. When indigodisulfonate or tetrasulfonate was similarly added, there was no observable change in color to indicate reduction by the cells (8), so that, when it caused increased respiration, there must have been some other reason than that it too was a reversible oxidation-reduction dye.

In further studying the effect of these dyes on the individual cell, they were added to dog's blood in the same concentration as to the liquid media in the respiration experiments in order to determine their effect on the cell counts. Up to 2 hours erythrocyte and leucocyte counts of the control and dye-added bloods had not been appreciably altered; but after 8 to 10 hours the leucocyte count of the methylene blue-added blood was from 1,000 to 2,000 less than the previous counts, whereas those of the control and indigosulfonate-added blood remained about the same as before. Whether the destruction of leucocytes was due to catalytic or other properties of methylene blue does not appear, but it does not seem probable that cells could continue to give up hydrogen to reduce methylene blue without suffering injury and in some cases extinction.

That methylene blue might have injured the cells of the tissues used in these experiments does not seem consistent with the fact that it also increased their respiration. Whether these two effects were compatible was studied by comparing the respiration of uninjured and x-ray injured Nereis eggs, Limulus heart, and rat liver. Each radiated rat received 2500 R over the liver area while alive, and later the liver was excised and its respiration measured as in the preceding experiments and compared with that of an unradiated litter mate. The Nereis and Limulus experiments were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts. Their respiration was measured by the method previously described, but at room temperature and with air and sea water as the gas and liquid media. Limulus heart survived excision well enough to continue beating for the forty hours covered by the experiments. One portion of the excised heart received 2500 R, and its respiration was compared with that of the unradiated portion. The Nereis eggs were divided into two portions, one of which was given 2500 R; both portions were fertilized, and their respiration then compared. In each case there

were 5 successive comparisons, and the value for each comparison was the average of several experiments. These averages are recorded in table 2 and show that the respiration of the radiated Nereis eggs, rat liver, and Limulus heart was immediately increased, but only temporarily, as it soon fell off to that of the unradiated tissue in each case. The comparisons were continued beyond the 5, but the values obtained have not been included in the table because their reliability was lessened by the greater experimental error involved as the respiration measurements became very small. The later comparisons seemed to indicate, however, that the respiration of the radiated tissues was then falling off faster than

TABLE 2

The effect of x-ray injury (2500 R) on the oxygen consumption of nereis eggs and excised rat liver and limulus heart

TIME AFTER X-RAY	AVERAGE OXYGEN CONSUMPTION (CU.MM./MGM./HR.) OF								
	Nereis eggs			Rat liver			Limulus heart		
	Number of experiments	Unradiated	Radiated	Number of experiments	Unradiated	Radiated	Number of experiments	Unradiated	Radiated
hours									
1	10	1.1	1.5				11	1.0	1.6
2	12	0.9	1.2						
3	11	0.7	0.9						
4	13	0.6	0.7						
5	15	0.5	0.5	11	12.1	15.4	12	0.9	1.4
10				12	12.3	15.2	12	0.8	1.2
20				11	12.2	15.0	13	0.6	0.8
30				14	12.2	13.9			
40				15	12.1	12.0	15	0.5	0.5

that of the unradiated. This was certainly true of the Nereis eggs, for the radiated eggs had ceased respiring with death, shortly after reaching the ciliated stage when the unradiated eggs were still alive and respiring. There could be no doubt that the radiated eggs had suffered injury, and that this injury had not prevented but was probably the cause of the temporary increase in respiration. It seems safe to conclude, therefore, that injury and increased respiration are compatible effects, and that the increased respiration caused by methylene blue under all the conditions set up in the foregoing experiments was probably due to injury rather than catalysis.

The effect of indigosulfonate, as remarked above, must have been on a different basis from that of methylene blue, for it was not observably reduced by the tissues and caused increased respiration only in the presence of calcium, or better, of calcium and phosphate. In order to study more fully this relationship between calcium and indigo as compared with

methylene blue, these dyes including 2 forms of indigo, disulfonate and tetrasulfonate, in the same concentrations as in the respiration experiments were added to a modified Ringer's solution containing 1 mM/L each of calcium and phosphate. Samples were drawn from the surface at once and at frequent intervals thereafter, and analyzed for calcium. In the indigodisulfonate-containing Ringer's solution the calcium concentration was fully maintained for 1 week and fell off but little during the subsequent weeks, whereas the calcium content of the control and methylene-blue-containing Ringer's solutions started to fall off at once and continued to fall off slowly during the period of observation. When equilibrium would be finally reached between calcium ions and precipitated calcium carbonate and calcium phosphate, the calculated calcium ion concentration would be of the order of 0.2 mM/L. The latter solution, when freshly made, is generally considered supersaturated with respect to calcium ions, but it would seem more correct to describe it as one in which the precipitation of $Ca_3(PO_4)_2$ is unusually slow. The indigodisulfonate Ringer's solution seems to come much nearer being a true supersaturated solution. The delay observed in the precipitation of calcium in this solution was probably due to the stabilizing effect produced by the competition of the several phosphate and sulfonate groups for calcium ions. On this basis the addition of more competing groups should serve to delay precipitation still further, and that is what was observed with the addition of tetrasulfonate instead of disulfonate. In such indigosulfonate-Ringer's solutions calcium ions probably exist in about the same state as in cerebrospinal fluid, and are probably more active than in either the slowly precipitating Ringer's or the citrate-Ringer's solution in which the calcium salts ionize to give 1 mM/L of calcium ions. Table 1 shows that respiration was at a maximum only in the presence of the presumably more active calcium ions of indigosulfonate-Ringer's solution and cerebrospinal fluid. It would seem, therefore, that indigosulfonate probably exerted its effect through a more active calcium probably exciting the oxygen present and rendering it more available for use by the tissue cells.

These results would suggest the possibility that such an indigo compound might serve a similarly useful function if present in the tissues. The indican found in human blood and urine is generally (9) attributed to intestinal putrefaction, but some (10) have considered that it results from intracellular protein metabolism. Hawk and Bergeim (9) say that bacterial decomposition of body protein, as in gangrene, putrid pus formation, etc., gives rise to an increased indican excretion. Wells (11) says that it is said that large amounts of indican may be excreted in cases of bulky secondary cancer of the liver without putrefaction. The possibility that the indican in all such cases might result from the existence of indigo in some form in the tissues involved led to an attempt to isolate it from normal rabbit tissues. The method followed was in essence that used

for plants (12, 13), modified to make it applicable to animal tissue. In spite of present crudities in the method, a product was obtained that gave the chemical tests (14) for indigotin, namely, a blue solution in chloroform or glacial acetic acid, reduction to the leuco form by hydrosulfite, and oxidation of the latter solution by air, regenerating indigotin. This product was obtained most abundantly from veins, but also from liver which is rich in veins. If indigo was present in arteries and striated muscle and the other tissues examined, it was in too small quantities to be isolated and detected by the methods which showed its presence in veins. It probably exists in the veins combined with sulfonate as part of a more complex molecule, and it may well be that it is this substance that imparts to veins their characteristic blue color.

The results of this study may be summarized as follows:

1. Variations in the constituents of the van Dyke-Hastings modification of Ringer's solution sometimes increased, sometimes decreased respiration, so that the average effect was generally nil.

2. Increases in respiration caused by the addition of methylene blue to the liquid medium may have been due to injury rather than to catalysis.

3. Respiration was at a maximum with a minimum of glycolysis in cerebrospinal fluid, probably on account of its calcium ion supersaturation.

4. The addition of indigodisulfonate or tetrasulfonate to Ringer's solution brought about a calcium ion supersaturation and respiration equal to those in cerebrospinal fluid.

5. Indigo was isolated from the veins of rabbits. Its function in the animal body might possibly be the maintenance in the body fluids of a state of calcium ion supersaturation, exciting the oxygen and making it more available for use by the tissues.

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DIETARY MANAGEMENT OF ALBINO RATS BEFORE AND AFTER THYROPARATHYROIDECTOMY

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Many investigators have observed the mortality of albino rats following thyroparathyroidectomy to be extremely variable. Several factors, such as environmental temperature, acidosis, infections, pregnancy, lactation gastro-intestinal irritation, constipation, oestrus, muscular exercise, menstruation and types of diet (1, 2, 3) have been found experimentally to influence parathyroprevic tetany and yet the accessory tissue hypothesis is commonly proposed to explain this variability. Although Hoskins and Chandler (4) by serial section of the neck were not able to find that more than 13 per cent of their animals possessed accessory parathyroid tissue, the accuracy of serial section technique to determine with absolute certainty the existence of accessory tissue cells is questioned by many investigators.

In our study of post-operative treatment the environmental temperature was kept constant, between 88 and 92°F. The rats selected varied in age from 24 to 33 days which effectively eliminated such possible interfering factors as oestrus, pregnancy and lactation. They were weaned at the age of 21 days and continued, until selected for the operation, on the stock diet which consisted of Fox Chow *ad libitum*, with bread, meat and cabbage once per week. In this manner variations which might result from different types of diet such as various degrees of growth and development and differences in the amount of food ingested preceding the operation were reduced to a minimum. Less controllable factors such as gastro-intestinal disturbances, low grade infections, muscular exercise and the individuality of the animal must be considered and some allowance made in accounting for experimental variations.

The importance of an intake of calcium above that in a normal diet has been stressed by several investigators (1). Our first work was designed to study the relative efficiency of certain diets in the prevention of tetany during the first 48 hours following thyroparathyroidectomy. Preliminary experiments (5) indicated that $Ca(OH)_2$ was superior as an inorganic salt to either the balanced salt mixture (Harris) or $CaCl_2$ in the prevention of parathyroid tetany during the first 48 hours after surgery.

For the first study 438 animals were divided into 6 groups (table 2). Group 1 (81 rats) received the Fox Chow diet. This diet was selected because it closely resembles stock diets in common use. Group 2 (76 animals) received a so-called standard diet, which could be easily modified (table 1), consisting of starch, casein, butter, yeast, cod liver oil, and an inorganic salt mixture (Harris). Group 3 (83 animals) received a modification of this diet made by substituting $CaCl_2$ for the balanced salt mixture. Group 4 (119 animals) received another modification of the standard diet in which $Ca(OH)_2$ was substituted for the salt mixture. In groups 5 and 6 the importance of NH_4Cl injections was studied. Group 5 (32 animals) received the $CaCl_2$ diet (same as group 3), while group 6

TABLE 1

	DIETS			
	Standard	$CaCl_2$	$Ca(OH)_2$	Salt deficient
Casein	18	18	18	18
Starch	47	45.5	47	50
Unsalted butter	15	15	15	15
Baker's yeast	15	15	15	15
Salts	3*	4.5†	3‡	0
Cod liver oil	2	2	2	2

* Balanced salt mixture (Harris).
† Anhydrous $CaCl_2$.
‡ $Ca(OH)_2$.

(47 animals) received the $Ca(OH)_2$ diet (same as group 4). Both of these groups received 3 subcutaneous injections of 0.3 mgm. of NH_4Cl per gram of rat. These injections were made at intervals of approximately 3, 8 and 20 hours following the thyroparathyroidectomy.

Since it was not practical to have litter-mate representatives of the same sex in all experiments, the animals in groups 1, 2, 3 and 83 of the animals in group 4 were so distributed as to have litter-mate representatives in each group. The remaining 36 in the 4th group and those of the 5th and 6th groups were distributed in the same manner. The sexes were equally divided in all experiments.

Shortly after thyroparathyroidectomy (within 1 hour) the animals were taken to the constant temperature room (88–92°F.) where they were kept for the following 48 hours. During the first 24 hours the animals were examined 3 times, and twice during the second 24 hours. At each examination the animals were classified as: surviving without tetany, surviving with tetany, or dead. An animal was considered as surviving with tetany if during the observation stiffening of one or more limbs

occurred, or if such a condition developed after one-half minute of exercise. Because of the difficulties in distinguishing mild degrees of tetany the number of deaths is obviously the most correct.

TABLE 2

DIVISIONS	DIETS	INJECT	NUMBER OF RATS		1ST 24 HOURS			2ND 24 HOURS			48 HOUR PERIOD		
					Surviving			Surviving			Surviving		
					Without tetany	With tetany	Dead	Without tetany	With tetany	Dead	Without tetany	With tetany	Dead
Part A													
Group 1	Fox Chow		81	Number	53	12	16	57	3	5	49	11	21
				Per cent	65	15	20	88	4	8	60	14	26
Group 2	Standard		76	Number	13	19	44	15	16	1	11	20	45
				Per cent	17	25	58	47	50	3	15	26	59
Group 3	$CaCl_2$		83	Number	27	10	46	33	2	2	27	6	50
				Per cent	33	12	55	89	6	5	33	7	60
Group 4	$Ca(OH)_2$		119	Number	83	12	24	86	3	6	81	8	30
				Per cent	70	10	20	91	3	6	68	7	25
Group 5	$CaCl_2$	NH_4Cl	32	Number	15	13	4	12	13	3	9	16	7
				Per cent	47	41	12	43	46	11	28	50	22
Group 6	$Ca(OH)_2$	NH_4Cl	47	Number	45	1	1	45	1	0	44	2	1
				Per cent	96	2	2	98	2	0	94	4	2
Part B													
Group 1	Fasting		96	Number	14	25	57						
				Per cent	15	26	59						
Group 2	Fasting	$NaHCO_3$	74	Number	5	29	40						
				Per cent	7	39	54						
Group 3	Fasting	NH_4Cl	75	Number	21	28	26						
				Per cent	28	37	35						

Per cent, approximate.
Age at operation, 24 to 33 days.

In tabulating the results, the observations of the first and second 24 hour periods were considered first separately and then as a unit. In the summary (table 2) an animal was recorded as being in tetany in the first

24 hour period if tetany appeared at either of the 3 observations. In the second 24 hours an animal was recorded as being in tetany if it appeared in either of the 2 observations during that period. Frequently an animal would be recorded as surviving with tetany in the first 24 hour period, and either dead or surviving without tetany in the second 24 hour period. Some animals which were recorded as surviving with tetany in the second period may have been recorded as surviving without tetany in the first period. Where the 48 hour period was considered as a unit, an animal was recorded as surviving with tetany if such was observed during any of the 5 examinations. During the first 24 hour period a low percentage of deaths occurred in groups 1 and 4 which received respectively the Fox Chow and $Ca(OH)_2$ diets. From 65 to 70 per cent of the animals in these groups survived without tetany.

In the groups receiving the standard (group 2) and the $CaCl_2$ (group 3) diets a high percentage of deaths was recorded while only 17 to 33 per cent survived without tetany.

In groups 5 and 6 where NH_4Cl injections supplemented the modifications of the standard diet the percentage of deaths was greatly decreased while the percentage of animals surviving without tetany was increased. In group 5, which received the $CaCl_2$ diet the percentage of deaths was 12 as compared with 55 in group 3 which received the same diet without the NH_4Cl injections. The percentage of animals surviving without tetany was 47 as compared with 33 in group 3. Although the NH_4Cl injections greatly decreased the percentage of deaths it did not increase the percentage surviving without tetany proportionately, thus leaving a high morbidity (41 per cent surviving with tetany).

In group 6, which received the $Ca(OH)_2$ diet only 2 per cent died as compared with 20 per cent in group 4 which received the same diet but no NH_4Cl injections. Ninety-six per cent of the animals survived without tetany in group 6 as compared to 70 per cent in group 4. The injection of NH_4Cl not only decreased the mortality but also the morbidity when $Ca(OH)_2$ was used as the inorganic dietary salt.

In the second 24 hours only a few animals died. The morbidity was much higher in the group receiving the standard diet than in the ones receiving either the Fox Chow or the modified standard diets. Of these groups the highest percentage of animals which survived without tetany received the $Ca(OH)_2$ diet. The lowest percentage surviving without tetany occurred in the group which received the standard diet.

Where the modified standard diets were supplemented by the injection of NH_4Cl no deaths occurred in the group which received $Ca(OH)_2$ (group 6) as the dietary salt. In group 5, in which the dietary salt was $CaCl_2$, the percentage of deaths was higher than in any of the other groups. The per cent surviving without tetany was even lower than during the first 24 hour period.

In summing up the results for the entire 48 hours it was observed that the Fox Chow and $Ca(OH)_2$ diets were more effective in preventing tetany and keeping a low morbidity than either the standard or $CaCl_2$ diets. Where NH_4Cl was used to supplement the $CaCl_2$ diet (group 5) the percentage of deaths was greatly decreased; however, the morbidity was increased because of the high percentage surviving with tetany and the low percentage surviving without tetany. In the group receiving $Ca(OH)_2$ (group 6) the mortality and morbidity was greatly decreased by the injection of NH_4Cl and 94 per cent survived without tetany.

The results of these experiments indicate that the condition of the animal following the removal of the parathyroids is largely dependent upon the type of inorganic salts supplied and the acid-base balance maintained. To further study the importance of the acid-base balance 245 animals were divided into 3 groups (table 2) and subjected to the same treatment and the same study as the previous groups except that no food was permitted following the operation. In group 2 (74 animals) 0.45 mgm. $NaHCO_3$ per gram of rat was injected while in group 3 (75 animals) 0.3 mgm. NH_4Cl was injected. Group 1 served as a control with 96 animals in which no injections were made. The duration of these experiments following the operation was only 24 hours. Extension through a second 24 hour period would probably incur other starvation factors independent of the relation to parathyroid deficiency. The injection of this quantity of $NaHCO_3$ was probably without a significant effect. The per cent surviving without tetany and the mortality was somewhat higher in the group injected with $NaHCO_3$. The mortality in the group receiving the NH_4Cl injections (group 3) was only 35 per cent while the mortality in the control group was 59 per cent. The percentage surviving without tetany was 28 compared to 15 per cent in the control group.

Having observed the great importance of post-operative treatment it seemed likely that pre-operative management would be equally important. The age of the animal at the time of the operation has been pointed out by various investigators (1) to be an important factor, the older the animals the less frequent the occurrence of tetany. An explanation of the importance of the age factor is more difficult than the observation. Since the older the animal is at the time of thyroparathyroidectomy the longer it has been on some type of pre-operative management—the larger the alimentary system for holding recently ingested material prior to the operation—the larger the skeletal system, and the greater the expansion of all tissues. A combination of these factors gives a greater possibility for the storage of material to be used in emergency.

The importance of pre-operative diets has been reported by us (6) in a preliminary paper. One hundred and thirteen animals were selected at the age of 28 days. Litter-mates of the same sex and as near the same

weight as practical were divided into 3 groups (table 3—A). The first group was continued on the stock diet (Fox Chow). The second group received the standard diet referred to in the first part of this paper. The third group received a modification of the standard diet in which no inorganic salts were added. These 3 groups were continued on their respective diets for 10 days at the end of which time they were thyroparathyroidectomized and subjected to a thermostatically controlled temperature,

TABLE 3

DIVISIONS	DIETS	DAYS ON DIET	NUMBER OF RATS	1ST 24 HOURS AFTER THYROPARATHYROIDECTOMY					
				Number			Per cent (approximate)		
				Surviving		Dead	Surviving		Dead
				Without tetany	With tetany		Without tetany	With tetany	
Part A									
Group 1	Fox Chow	10	33	5	8	20	15	24	61
Group 2	Standard	10	39	2	15	22	5	39	56
Group 3	Salt deficient	10	41	0	1	40	0	2	98
Part B									
Group 1	Fox Chow	100	38	19	18	1	50	47	3
Group 2	Standard	25	13	6	6	1	46	46	8
Group 3	Salt deficient	25	16	1	11	4	6	69	25
Group 4	Standard	50	12	4	7	1	33	59	8
Group 5	Salt deficient	50	24	0	4	20	0	17	83
Group 6	Standard	75	54	12	30	12	22	56	22
Group 7	Salt deficient	75	46	0	2	44	0	4	96
Part C									
Group 1	Standard	50	11	7	4	0	64	36	0
Group 2	Salt deficient	50	12	1	11	0	8	92	0

Age at operation: Part A, 38 days; Part B, 100 days; Part C, 150 days.

88–92°F., without food for 24 hours. In this manner the importance of pre-operative dietary management for a few days prior to the operation could be studied uncomplicated by the variable appetites and food ingested following the operation. The animals were inspected 3 times as previously described. The mortality of the groups (group 1 and 2) which had previously received Fox Chow or the standard diet was 56 to 61 per cent. The percentage surviving without tetany was somewhat higher in the group receiving Fox Chow. In the group receiving the salt de-

ficient diet the mortality was 98 per cent and the morbidity was 100 per cent. Thus the importance of the dietary management in even a short pre-operative period is shown. If the importance of the age of the animals is dependent upon the factors which we have suggested, it should be necessary to keep older animals for a longer period of time on a dietary deficiency than was required when dealing with 28 day old animals in order to obtain comparable results.

This study was then extended to include animals thyroparathyroidectomized at the age of 100 days which had been on different types of pre-operative dietary management for varying periods of time (table 3—B). Two hundred and three animals were divided into 7 groups. The first group (38 rats) served as a control and received the Fox Chow diet throughout the 100 day period. The 2nd and 3rd groups (13 and 16 animals respectively) received the Fox Chow diet until 75 days of age after which group 2 received the standard diet and group 3 received the salt deficient diet for the succeeding 25 days. The 4th and 5th groups (12 and 24 animals respectively) received the Fox Chow until 50 days of age after which group 4 received the standard diet and group 5 the salt deficient diet for the succeeding 50 days. Groups 6 and 7 (54 and 46 animals respectively) received Fox Chow until 25 days of age after which group 6 received the standard diet and group 7 the salt deficient diet for the succeeding 75 days. The animals were so distributed as to obtain litter-mate representatives of the same sex in each group. The results of these experiments revealed that animals kept until the age of 100 days on a Fox Chow diet were very resistant to the parathyroprevic state even though they were kept in a fasting condition for 24 hours following the operation. Fifty per cent of the animals survived without signs of tetany and only 3 per cent died. A comparison of the morbidity and mortality in those groups which received the standard and salt deficient diets for variable periods of time reveals neither of these diets as efficient as Fox Chow, however, the standard diet was much more efficient than the salt deficient diet. A mortality of 83 per cent and a morbidity of 100 per cent was obtained following thyroparathyroidectomy at the age of 100 days if during the 50 days immediately preceding the operation, the animals received the salt deficient diet. These results are comparable to the results obtained on 38 day old animals which had received the salt deficient diet for only 10 days. Increasing the salt deficiency period to 75 days preceding the operation (group 7) raised the mortality to 96 per cent.

To further study the effect of salt deficiencies at different ages 23 animals, litter-mates to the animals in the previous groups were divided into 2 groups at the age of 100 days (table 3—C). Group 1 (11 rats) received the standard diet and group 2 (12 rats) the salt deficient diet for the succeeding 50 day period. While no mortality occurred in either group 92

per cent of those on the salt deficiency survived with tetany as compared to 36 per cent in the group receiving the standard diet. Sixty-four per cent of the animals in the latter group survived without tetany while only 8 per cent of the group on the salt deficiency survived without tetany. The results of group 2 in this series when compared with the results obtained on group 5 of the preceding series which received the salt deficient diet for a comparable period of time, is further evidence that pre-operative management is far more significant in the younger animal.

Since proving or disproving the presence of accessory parathyroid tissue is difficult, and since it is even more difficult to prove the function of such tissue, it becomes important to determine the necessity of relying upon the accessory tissue hypothesis to account for the survival of some animals for a greater period of time than others after parathyroidectomy. Evans et al. (7) have expressed the opinion that the presence of accessory parathyroid tissue does not explain the survival of all parathyroidectomized dogs, since parathyroidectomized dogs may survive for at least 9 months with low serum calcium and high inorganic phosphorus. After studying the importance of environmental temperature and of returning the animals immediately to an adequate diet following the operation in previous work we expressed the view that the accessory tissue hypothesis is not necessary to explain variabilities (8). The present work seems to confirm this idea. When the proper account of all controllable variables is taken into consideration and sufficient allowance is made for certain uncontrollable factors the variabilities in mortality and morbidity following thyroparathyroidectomy seem adequately explained.

SUMMARY

1. $Ca(OH)_2$ is superior to $CaCl_2$ as an inorganic dietary salt for the prevention of parathyroid tetany in the rat during the first 48 hours following thyroparathyroidectomy.

2. The injection of NH_4Cl decreases the mortality and morbidity following thyroparathyroidectomy when rats are kept in a fasting state.

3. The injection of NH_4Cl into thyroparathyroidectomized rats receiving $CaCl_2$ and $Ca(OH)_2$ as the source of inorganic salts greatly improves their conditions.

4. Depriving young animals of adequate salt intake for only a few days prior to thyroparathyroidectomy increases the mortality and morbidity.

5. The older the rat at the time of thyroparathyroidectomy the more resistant it is to a period of pre-operative salt deficiency.

6. Since it is necessary to control several factors following thyroparathyroidectomy of albino rats it seems likely that the variability in mortality

and morbidity often obtained is due to one or more of these factors rather than to accessory parathyroid tissue.

This work was conducted under the general supervision of Dr. A. J. Carlson to whom we are greatly indebted.

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THE LIFE CYCLE OF THE RED BLOOD CELL IN THE DOG

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Even a cursory examination of the literature will reveal great divergence of opinion as to the life cycle of the red blood cell. Textbooks usually give 30 days as the life of the red cell in the circulation but it is not difficult to marshal evidence to fix this period as 15 days or any interval up to 100 days. This laboratory has a legitimate interest in this question as the normal wear and tear or wastage of red cells is of distinct importance in any study of anemia and hemoglobin regeneration.

There are obvious flaws in the experimental techniques used by the host of workers in this field. Probably the most accurate work has been done in human beings with transfusion of red cells of one group into a recipient of a different group (1, 13). But one transfuses cells of all ages and only the new cells which perish at the end of the cycle will give results of value. This point is difficult to determine and furthermore the patients are in some respects abnormal. However these results are closer to the figures given below than any others recorded in the literature. It is possible of course that animals may differ among themselves and from human beings in respect to the life of the red cell in the circulation.

The bile fistula in the dog has been used in this laboratory for many years to study pigment metabolism and much has been learned about the related clinical abnormalities (5) and how they may be controlled. By methods described (5) we are able to keep certain bile fistula dogs in perfect health, normal activity, weight equilibrium and appetite for many years. We would stress this *normal state* of the bile fistula dog and attach little significance to observations made upon bile fistulas which present weight loss, lack of appetite or bleeding. These normal dogs eliminate quantitatively the pigment radicle and when 1 gram of hemoglobin is given intravenously we recover 40 mgm. of bile pigment above the control level (12). If considerable amounts of red cells should go to pieces in the circulation during any given period, the resultant increase in bile pigment should indicate the amount of the destroyed red cells.

Given a normal bile fistula with great numbers of *new red cells* in the circulation the bile pigment output should be low until these cells reach

maturity and disintegrate. At that time there should be a sharp rise in the output of bile pigments. This is precisely what happens as is seen in the charts below. The circulation of these bile fistula dogs is filled with new red cells by rapid hemoglobin and red cell regeneration following bleeding or blood destruction. These new red cells are turned into the circulation within a period of 2 to 4 weeks and we may take the mid point of this period as representing the average starting point. Then the end point is fixed as the peak of the increase in bile pigment excretion. Between these two points the bile pigment excretion is low. The time elapsed between the starting and end points averages 124 days with specific values of 112, 120, 126 and 133 days. As physiological experiments go, this represents a considerable degree of uniformity.

Ashby (1) as the result of studying the length of time transfused red cells may persist in the recipient arrived at a life cycle of from 30 to 100 days with an average stay of 83 days. Wearn, Warren and Ames (13), using the same method, found the transfused cells persist from 59 to 113 days. The patients were all abnormal due to either primary anemia or some form of secondary anemia. Isaacs (6) gives no figure but thinks transfused cells last only a short time, a few days as a maximum.

Kurtz (9) on the basis of reticulocyte counts in rabbits estimates 42 days as the average length of red cell life with variation from 16 to 73 days. In contrast Eaton and Damren (3) on the basis of reticulocyte studies following hemorrhage in rabbits concluded the cells exist for only eight days.

Derom (2) estimates 15 to 20 days as the life cycle for dogs on the basis of elimination of transfused cells. Escobar and Baldwin (4) estimate the red cell life to be 12 to 18 days in rats; 16 to 23 days in dogs and 18 to 30 days in man. They caused an increase in erythrocyte volume in circulation by short exposure to low pressure of oxygen and consider the number of days elapsing from end of exposure period to attainment of normal red cell volume as an indication of duration of life of the red cell.

Methods. Two types of fistula dogs were utilized: the gall-bladder renal type of Kapsinow, Engle and Harvey (7) in which the gall bladder is fixed in the renal pelvis, the common bile duct obstructed and the bile flows freely into the ureter with total collection in the urine; and the closed sterile fistula as devised by Rous and McMaster (11) in which the total bile is collected in a sterile bag. The methods used in these experiments and care of these animals have been adequately described in a previous paper (8).

The salmon bread diet consists of salmon bread, salmon, and "Klim" (a commercial skim milk powder) mixed with water to form a mash. The preparation of the salmon bread has been described (15). The kennel diet is a mixture of hospital kitchen scraps. Both of these diets are rich

in carbohydrates and low in fat content which renders them suitable for fistula dogs.

These dogs can be kept in excellent clinical condition free from the intoxicated states resulting from bile deprivation provided they are fed suitable food and are given an adequate amount of bile (50 to 75 cc.) by mouth daily. This bile by mouth does not complicate bile pigment excretion studies since bile pigments are not reabsorbed from the intestines and resecreted as such, as is true for bile salts.

The plan of the experiments is simple. An adequate base line for bile pigment elimination and circulating hemoglobin is obtained during a prolonged fore period. The dogs are then made anemic either by bleeding or by means of blood destruction caused by acetyl phenylhydrazine. They are allowed to recover from the anemia and pigment studies are continued through the after period. Daily analyses of total bile pigment elimination per 24 hours are made and hemoglobin determinations made each week. The hemoglobin base line is fixed at 13.8 grams equivalent to 100 per cent.

Acetyl phenylhydrazine proved more satisfactory in causing the anemia as the dogs recovered from the anemia much more promptly and were back to the normal level within two weeks. This is due to the fact that the iron and presumably the globin from the destroyed cells are retained in the body and can be utilized in the prompt formation of new hemoglobin. It seems probable that the hydrazine destroys the older and presumably less resistant red cells.

It is dangerous to catheterize these dogs frequently. Therefore in the renal bile fistulas the 24-hour urine specimens are collected from the cage for bile pigment analysis. The levels and figures given in charts and tables are averages of 7 days and in this way the cage specimens approximate closely to the actual catheterized values as bladder urine retained on one day will appear in the next day's collection.

Experimental observations. In the first experiment carried out on dog 31-351, a mongrel hound, the *anemia* was produced by *repeated bleedings*. This dog had carried a *gall-bladder renal fistula* for fifteen months and during this period had been in excellent clinical condition maintaining an average weight of 18 kilos. There had never been any signs of intoxication due to bile deprivation. It had been fed the salmon bread diet with the occasional addition of cooked pig liver. Ox bile 50 cc. was mixed with the food daily. The chart shows the circulating hemoglobin percentage and the daily average amount of bile pigment eliminated per week. It required 11 bleedings during a four week period to lower the hemoglobin from the level of 123 per cent (17 grams) to 43 per cent (5.9 grams). The blood removed amounted to 2380 cc. and it contained 197 grams of hemoglobin. Normally this dog had a blood volume of 1650 cc. and an average circulating hemoglobin of 120 per cent (16.5 grams) or about 270 grams of

hemoglobin in its circulation. At the height of the anemia, at most, there would be only 73 grams of *old hemoglobin* left in the body. There would actually be less, since during the four week period of bleeding some new cells would have been formed and in part removed in the later bleedings.

Subsequent to the bleeding period, cooked pig liver (200 grams) and Lextron 6 grams (a mixture of iron citrate plus a potent liver fraction active in anemia due to blood loss (16)) were added daily to the salmon bread diet in order to expedite new hemoglobin formation. However, it took four weeks for the hemoglobin to return to 100 per cent (13.8 grams) at which level it remained with but slight variations. As the anemia was produced the bile pigments gradually decreased from the initial level of 135 mgm. a day, finally reaching a low point of 78 mgm. The amount eliminated remains lower than normal for several weeks, and then increases gradually

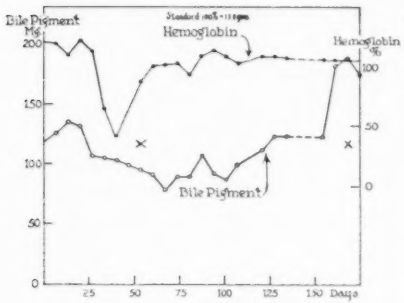

Chart 1

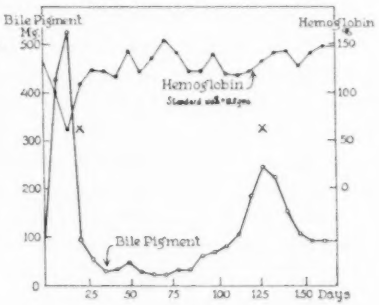

Chart 2

Chart 1. Dog 31-351. Life cycle of red cell = 120 days
Chart 2. Dog 34-212. Life cycle of red cell = 112 days

with finally an abrupt increase to 181 mgm. per day. The dotted line indicates a three week period when pigment determinations were not made.

In estimating the life cycle of the red cells one might choose as the starting point, the time when the blood hemoglobin was at its lowest point as from experience we know that during the four week bleeding period many new red cells have been formed and liberated into the blood stream. As the end point we have taken that period when the bile pigment output was greatest since it is during this period that disintegration of red cells is at its maximum. This interval equals 133 days. If we take as a starting date the *mid point* in the curve of most active hemoglobin regeneration, the life cycle is fixed at 120 days. This corresponds to the starting point in the next three experiments.

On the basis of a 120 day cycle this dog destroys 0.83 per cent of its blood hemoglobin daily. It had a circulating hemoglobin of 270 grams,

so 2.24 grams of hemoglobin should be destroyed and this is equivalent to 90 mgm. of bile pigment. During a basal period of 63 days before the experiment, the daily average of bile pigment excreted was 112 mgm.

Dog 34-212, a mongrel hound, was a *closed sterile bag fistula* of eight months' standing, and had been fed kennel diet 500 to 600 grams mixed with ox bile 100 cc. daily. The clinical condition had been excellent with gain in weight from the operative level of 11.8 kgm. to 17.7 kgm. The dog was thin, but active and normal at the time of the operation. This is evidence that the dog was tolerating the fistula without clinical abnormality. The *anemia* was produced by *acetyl phenylhydrazine* given subcu-

TABLE 1

Bile pigment elimination

WEEK	WEEKLY TOTAL	DAILY AVERAGE	HEMOGLOBIN LEVEL AVERAGE	
	mgm.	*mgm.*	*grams per cent*	*per cent*
Control	649	93	18.4	133
9th	160	23	18.6	135
10th	158	22	21.0	153
11th	228	32	19.6	142
12th	225	32	16.8	122
13th	319	45	17.0	123
14th	431	61	18.9	137
15th	491	70	16.3	118
16th	567	80	16.1	117
17th	748	107	16.8	122
18th	1284	184	18.2	132
19th	1690	241	19.2	139
20th	1570	224	19.6	142
21st	1063	152	17.5	127
22nd	743	106	19.3	140
23rd	654	93	20.0	147

taneously on successive days in five doses of 100 mgm. dissolved in 10 cc. of water. The hemoglobin fell from 133 (18.4 grams) to 59 per cent (8.2 grams) within the week followed by prompt recovery from the anemia. The hemoglobin returned to 108 per cent (14.9 grams) in nine days, and at the end of the next week it was 124 per cent (17.1 grams). The hemoglobin subsequently fluctuated between 124 (17.1 grams) and 150 per cent (20.1 grams). As the red cells were destroyed, there was rapid increase in the bile pigments with 5333 mgm. eliminated above control amounts in a two week period. This is equivalent to 133 grams of hemoglobin destroyed (40 mgm. bile pigment = 1 gram of hemoglobin). As soon as the excess pigment resulting from the destroyed cells was eliminated, the bile pig-

ment excretion fell to very low levels, only 22 mgm. a day. For eight weeks this low level was maintained and then there was a gradual increase until finally there was an abrupt increase to 240 mgm. a day. Since, during the first week after the anemia there was a great production of red cells, we have chosen the end of this week as the starting point, and as the end point that period during which there was the greatest bile pigment output. The time elapsed between these points is 112 days.

In the after period when the new formed red cells were disintegrating, a period of 8 weeks, 6891 mgm. of bile pigment were eliminated above the amount excreted during the eight weeks just preceding when the output was low. This is equivalent to 172 grams of hemoglobin as compared with 133 grams destroyed originally by the drug.

The dog had a blood volume of 1570 cc. and an average hemoglobin of 130 per cent (17.95 grams) or a circulating hemoglobin of 282 grams. A life cycle of 112 days means that this animal should destroy 0.89 per cent of its blood hemoglobin daily, or 2.5 grams. The theoretical yield of bile pigment is 100 mgm. a day, whereas actually 91 mgm. were eliminated during control periods, totalling 53 days taken before and after the experiment.

Table 1 (dog 34-212) is included in order to illustrate the abrupt increase in the bile pigments as the new cells become of age and finally disintegrate. The weeks are numbered from the beginning of the experimental period. The very low output during the 9th to 13th weeks inclusive is of interest when one remembers that the dog had at this time a normal circulating hemoglobin volume. The greater part of this hemoglobin was in new cells, and the pigment that is being eliminated comes from hemoglobin of the older cells as they disintegrate.

Dog 31-271, a mongrel bull, had a *renal fistula* for three months and had maintained an average weight of 13 kgm. It had been fed the salmon bread diet mixed with dog bile 50 cc. daily.

As the result of eight doses of acetyl phenylhydrazine, 100 mgm. given daily subcutaneously, the hemoglobin fell from 127 (17.5 grams) to 50 (6.9 grams) per cent. Excess bile pigment resulting from red cell destruction amounted to 4057 mgm. which is equivalent to 101 grams of hemoglobin. In two weeks the hemoglobin increased from 50 (6.9 grams) to 100 per cent (13.8 grams), and in the following weeks there was additional increase to as high as 157 per cent (21.6 grams). After the excess bile pigment had been eliminated, the pigment output fell from the initial control level of 61 mgm. to the low level of 38 mgm. in a few days, and it remained low for nine weeks, and then gradually rose to 117 mgm. During the after period from the 17th to 26th weeks, 3109 mgm. of bile pigment were eliminated over the amount excreted during the 7th to 16th weeks immediately preceding, when bile pigment output was low. This is equivalent to 77 grams of hemoglobin as compared with 101 grams origi-

nally destroyed. In this dog the life cycle of the red cell is estimated as 126 days.

The blood volume was 1150 cc. and the average circulating hemoglobin was 125 per cent (17.25 grams) or 198 grams of hemoglobin. With a cycle of 126 days, the dog destroys 0.79 per cent of its red cells and hemoglobin daily, or 1.56 grams, so theoretically 63 mgm. of bile pigment should be eliminated. Actually, an average of 66 mgm. is recovered in control periods equalling 100 days taken before and after the experiment.

Dog 34-211, a mongrel hound, had had a *renal fistula* of ten months' duration when this experiment was performed. On the diet of salmon bread plus ox bile 50 cc. daily it had maintained an average weight of 18.3 kgm.

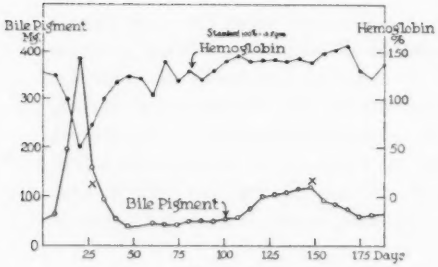

Chart 3

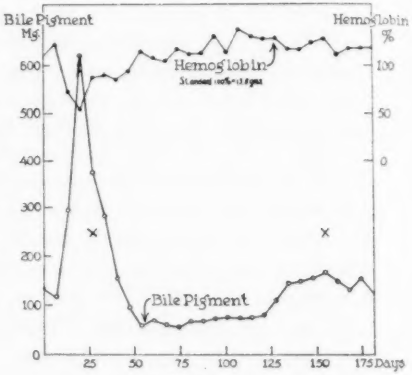

Chart 4

Chart 3. Dog 31-271. Life cycle of red cell = 126 days
Chart 4. Dog 34-211. Life cycle of red cell = 133 days

Eleven injections of *acetyl phenylhydrazine* in 100 mgm. doses spread over 14 days were necessary to lower the hemoglobin from 120 (16.6 grams) to 54 per cent (7.5 grams). As a result of the red cell destruction, 7034 mgm. of excess bile pigment equivalent to 176 grams of hemoglobin were eliminated. The bile pigment output decreased to an average of 58 mgm. per day as compared with the initial level of 125 mgm. After 10 weeks, the output increased until the peak level of 166 mgm. was reached. The life cycle of the red cell is estimated as being 133 days in length.

In the after period during the 19th to 27th weeks, 4255 mgm. of bile pigment were excreted in excess of the amount eliminated during the 9th to 18th weeks of reduced output. This is equivalent to 104 grams of hemoglobin or 72 grams less than the amount originally destroyed.

On the basis of a 133 day cycle, this dog destroys 0.75 per cent of its red cells daily. With an average blood volume of 1550 cc. and a circulating hemoglobin of 120 per cent (16.55 grams), the dog would have 256 grams of circulating hemoglobin. Consequently, 1.92 grams of hemoglobin would be destroyed daily, and the theoretical amount of bile pigment to be eliminated would be 77 mgm. During a control period of 31 days before the experiment, the average amount excreted was 72 mgm.

Discussion. It may be wise to emphasize again the importance of a *normal clinical state* in the bile fistula dogs as of great importance in these experiments. Abnormalities of various sorts may disturb the regular elimination of bile pigments in these fistula dogs and presumably the same may occur in abnormal human beings. It may be objected that even these clinically normal dogs are physiologically abnormal because the bile is excluded from its normal flow into the gastro-intestinal tract and bile given only by mouth. With this objection in mind one could hardly accept this argument as evidence that red cells in bile fistula dogs would tolerate circulatory vicissitudes for an abnormally long time. If these dogs are slightly abnormal because of the bile fistulas the life cycle of the red cell presumably would be if anything slightly shorter than in the absolutely normal non-fistula dog. We believe these figures approximate closely the actual life cycle of the red cell in the normal dog.

Table 2 summarizes the more important results of the four experiments. The actually observed *daily bile pigment elimination* during long control periods averages 85 mgm. per 24 hours in the four dogs. The *estimated bile pigment* elimination accounted for by red cell obsolescence (as determined by the life cycle of the red cells) averages 83 mgm. per 24 hours in the same dogs. If this is a coincidence it is remarkable and as physiological experiments go, it is sufficiently unusual to excite comment. Individual dogs vary somewhat as is perhaps not surprising. The calculated bile pigment at times exceeds the actual measured bile pigment and vice versa.

Perhaps it is to be expected that pigment elimination may vary somewhat from one period to another and perhaps at times relate to other factors than red cell hemoglobin. *Muscle hemoglobin* must be mentioned at this point in the discussion for it has been established (14) that when muscle hemoglobin is introduced into the circulation bile pigment is promptly formed. How rapid is the wastage of muscle hemoglobin in the body and what are its end products? Does muscle hemoglobin contribute to the eliminated bile pigment? It must be accepted that under certain conditions (myositis) the muscle hemoglobin may contribute to bile pigment formation but we do not know whether this obtains under normal conditions. After periods of long exercise there is evidence (10) that bile pigments may be increased and one may choose to believe that the muscle

hemoglobin has been used and wasted. It may be argued on the contrary that the red cells are traumatized by the prolonged exercise and account for the increased bile pigment output.

Muscle hemoglobin is incorporated in the striated muscles to serve some useful purpose and its mass is considerable—in fact, it may amount to as much as the circulating hemoglobin in anemia (16). Obviously it is not immortal and its waste products might include bile pigment. One may argue that the muscle fibers replace and repair their own proteins including muscle hemoglobin from protein stores, that this reaction takes place *within* these muscle fibers and that the pigment radicle does not escape. This whole paragraph is a confession of ignorance but indicates how badly we need more information.

TABLE 2

DOG NUMBER	RED CELL LIFE CYCLE	ESTIMATED BILE PIGMENT	ACTUAL OBSERVED BILE PIGMENT	BASAL CONTROL PERIOD	WEIGHT
	days	*mgm. per 24 hrs.*	*mgm. per 24 hrs.*	*days*	*kgm.*
31-351	120	90	112	63	18
34-212	112	100	91	53	17
31-271	126	63	66	100	13
34-211	133	77	72	31	18
Average....	124	83	85		

SUMMARY

Given a bile fistula dog in a normal state of health, weight equilibrium, appetite and activity we observe a uniform sustained output of bile pigment.

By blood destruction or blood withdrawal we can force this dog to fill its circulation with a great mass of *new* red cells and hemoglobin.

Following this procedure the bile pigment output will fall to low values for many weeks but subsequently there will be a conspicuous rise in bile pigment output. This rise marks the obsolescence of these new red cells and determines the length of their life cycle in a normal bile fistula dog.

These figures in four different dogs are 112, 120, 126 and 133 days—an average of 124 days. On the basis of these figures the *calculated* output of bile pigment averages 83 mgm. per day and the *observed* output averages 85 mgm. bile pigment per day.

Under these conditions the life cycle of red blood cells in the normal dog approximates 124 days.

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THE ACTIVITY OF THE BLOOD SERUM AMYLASE IN THE HYPOPHYSECTOMIZED DOG

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Changes in the blood serum amylase activity have been demonstrated in acute pancreatitis in the human and in various experimental procedures involving the pancreas. Summaries of the literature on serum amylase function are available in the recent papers of Elman (1), and Friedman and Thompson (2). The accepted opinion by workers in this field is that changes in blood serum amylase activity are due to alterations in the function of the externally secreting cells of the pancreas. The source of the blood serum amylase remaining after pancreatectomy, however, has not been satisfactorily explained. Markowitz and Hough (3), and Reid, Quigley and Myers (4), have shown that the lowered serum amylase activity following pancreatectomy is raised to normal by insulin administration. This points to an intimate relationship between insulin function and serum amylase activity. It is possible that at least some of the changes reported following pancreatitis and experimental procedures involving the pancreas might be due to changes in insulin balance.

The anterior pituitary is also intimately related to internal carbohydrate metabolism and is in general antagonistic to the action of insulin. It was decided, therefore, to determine what rôle the anterior pituitary might play in relation to serum amylase activity.

METHODS. The dog was used in these experiments.

Since serum amylase has not been isolated, only its activity can be measured. A change in activity may represent either an actual change in quantity of the active enzyme or a change in substances, other than the actual enzyme, which inhibit or activate the amylase reactions. Serum amylase activity was measured by a modification of the method of Scharles and Salter (5). This method measures loss of glycogen rather than the reducing sugar produced during a period of amylase activity. Details of the procedure for dog blood serum will be published elsewhere (6).

Rabbit liver glycogen was used (7). All determinations were made for three hours at 43°C., buffered at pH 7.0. For the different serum concentrations used the final glycogen dilution was 1 per cent, the solution volume 2 cc.

TABLE 1

Serum amylase activity in terms of glycogen loss before and after hypophysectomy

DOG NUMBER	WEIGHT	PREOPERATIVE		POSTOPERATIVE												CAUSE OF DEATH	ADDITIONAL STUDIES	
		Average	Immediate	Day 1	Day 2	Day 3	Day 5	Day 10	Day 15	Day 20	Day 30	Day 40	Day 50	Day 60	Day 70		Preoperative	Postoperative
	kgm.																	
1*	7.0	11.1	10.6	12.4	12.7		11.9		11.6	13.7	11.3	12.2	11.1	12.6			Food fast Pregnancy	Pregnancy Whelping Lactation Weaning
		7.5	*7.1*	*8.5*	*8.9*		*6.8*		*7.7*	*9.0*	*7.2*	*8.2*	*6.8*	*9.0*				
2	14.5	10.4	9.3		16.4		18.3		17.9	16.4	15.4	Died				Lobar pneumonia	Food fast Glucose Insulin Adrenin	Glucose Adrenin
			5.7				*12.9*		*13.1*	*11.2*	*12.0*							
3	19.0	12.2	11.2	Died												Bronchopneumonia	Food fast Glucose Insulin Adrenin	
4	7.5	11.2	11.2		16.8		18.9	18.0	17.0	16.8	17.2	17.9	17.4	16.2	17.5	Hypoglycemia		Glucose Adrenin
										11.6	*12.1*	*13.2*	*12.9*	*10.0*	*12.5*	Died 72 days p-o		Hypoglycemia
															Died			
5	14.0	14.5	16.6	16.9	19.4			Died								Hypoglycemia		
6	10.0	14.2	14.2		19.2		18.8 Died									Hypoglycemia		
7	8.5	12.1	12.1	14.3	16.8	15.4	16.2	16.4	Died							Hypoglycemia		

* Control.

Resting, fasting and non-fasting, values only are included. No values following injections of glucose, insulin, adrenin, are given. Figures in ordinary print represent 5 per cent serum concentration; italicized, 2.5 per cent serum concentration. Results indicate glycogen loss from the 20 mgm. in substrate solution.

The figures and charts are based upon the amount of actual glycogen lost during a period of activity.

Hypophysectomy was carried out by the approach through the floor of the sella turcica (8). Careful gross observation and the occurrence of spontaneous hypoglycemia were used to determine the completeness of hypophysectomy.

Within the first forty-eight hours postoperatively, each animal was given 1 to 2 cc. of pituitrin subcutaneously to diminish the initial polydipsia.

The control operation was done to eliminate anesthesia, trauma to the salivary glands, sepsis, and pituitrin, as a cause of the positive findings. The operation was a complete duplicate except that the pituitary body was not disturbed.

EXPERIMENTS. The experiments were divided into three parts. Seven dogs in all were used. The serum amylase activity determinations are summarized in table 1.

1. Control operation. Dog 1 was pregnant when the control operation was performed. Before operation the effect of fasting and food was observed. She was whelped on the tenth postoperative day of normal puppies, lactated until the forty-seventh postoperative day when the puppies were weaned. All observations were within the limits of normal dog serum amylase activity.

2. Other controls. The effects of: 1, forty-eight hour fast; 2, mixed diet; 3, glucose, 1.5 grams per kilogram and 4.5 grams per kilogram; 4, intravenous insulin, 2.6 units per kilogram, and 5, subcutaneous adrenin,[1] 0.1 mgm. per kilogram, were studied in dogs 2 and 3 before hypophysectomy. The effect on the serum amylase activity and blood sugar is shown for dog 2 in chart 1. Almost identical effects were produced in dog 3 and are, therefore, not shown. With the exception of the results following adrenin, all observations on serum amylase activity fell within the expected limits for the normal dog. There was a suggestive rise following adrenin administration. Cohen has reported a drop in serum amylase activity following adrenin (9).

3. Effect of hypophysectomy. Dogs 2 to 7 were hypophysectomized. In dog 4 a portion of the posterior lobe was left intact, attached to the posterior clinoid process. Total hypophysectomy was performed in the remainder. Dog 3 died thirty-six hours after operation of bronchopneumonia, and no postoperative data are included. Autopsy in all of these animals revealed complete removal of the pituitary (except in dog 4), and all the expected changes in the body as a whole.

The dogs observed following hypophysectomy showed an abrupt rise in serum amylase activity within the second twenty-four hours. This

[1] Parke, Davis and Co. 1:1000 adrenalin hydrochloride solution.

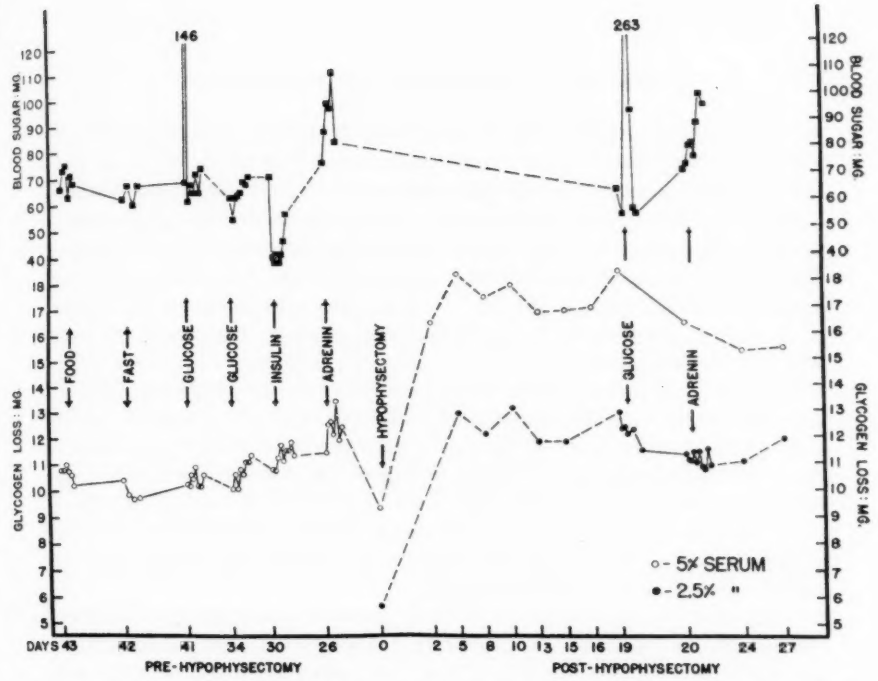

Chart 1. Dog 2, female, weight 14.5 kgm. Normal control and post-hypophysectomy studies: amylase activity, 2.5 per cent and 5 per cent serum, and blood sugar studies.

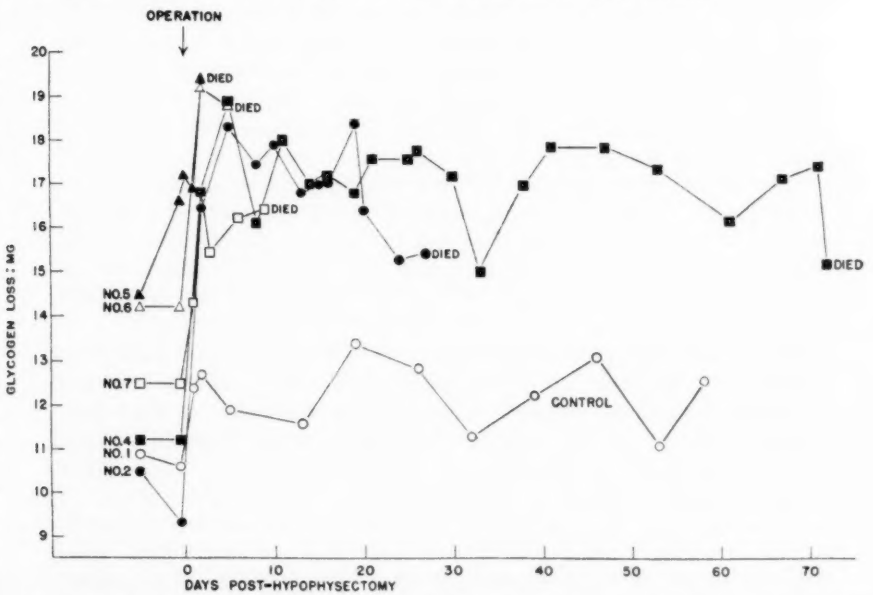

Chart 2. Combined chart of all animals except dog 3, amylase activity, 5 per cent serum.

elevation to abnormally high activity was sustained until death in all animals. The values reached in all animals were higher than any values seen in all the normal controls with the exception of a single observation in dog 5. In this animal the immediate preoperative value was higher than we have encountered in any other normal dog, and following hypophysectomy the serum amylase activity rose in forty-eight hours to the highest reading observed in any animal. This reading was at the upper limit of the experimental method; nearly the entire amount of glycogen present had been broken down.

In the second half of chart 1 are shown the data following hypophysectomy in dog 2. Two and five-tenths per cent serum after hypophysectomy showed in general a slightly greater serum amylase activity than 5 per cent serum before operation. Glucose and adrenin administered following hypophysectomy showed no significant variations in serum amylase activity although the expected blood sugar changes were found. In dog 4 after hypophysectomy, glucose produced no change but adrenin caused a slight rise in serum amylase activity.

Chart 2 is a composite graph of all 5 per cent serum amylase activity determinations following hypophysectomy together with the postoperative figures of the operative control. On the seventieth day dog 4 was allowed to go into spontaneous hypoglycemia. For twenty-four hours, from the seventy-first to the seventy-second day, the animal was prostrated, ending in terminal convulsions. The blood sugar ranged from 33 mgm. to 27 mgm. During this period of hypoglycemia there was a drop in serum amylase activity but the level remained within the variations previously observed in this animal since hypophysectomy. The spontaneous hypoglycemia observed following the diminished carbohydrate intake is physiological proof of the absence of anterior pituitary tissue.

Discussion. The significance of the enzyme amylase in blood serum is not known. That it is in some manner related to endocrine control of carbohydrate metabolism was suggested by the drop in activity following pancreatectomy and a return to normal with insulin administration reported from other laboratories. From the experiments quoted in this paper it is obvious that the pituitary exerts a profound influence.

Scharles and Salter give a formula for calculating an alpha value, the equivalent of amylase activity. Originally calculated for rat liver and tumor amylase, our experiments suggest that it also holds for dog serum amylase activity. If this formula is applied, following hypophysectomy there is more than a twofold increase in amylase activity. The increase in activity may be judged in another manner. In table 2 are given the values of glycogen loss resulting from different concentrations of normal dog blood serum, ranging from 2.5 per cent to 15 per cent serum. The glycogen loss from 5 per cent serum after hypophysectomy is approxi-

mately equivalent to that from 10 per cent to 15 per cent serum of the normal dog. Also, as pointed out, in chart 1, 2.5 per cent serum after hypophysectomy showed in general a greater glycogen loss than 5 per cent serum before operation.

The rise in serum amylase activity following the removal of the anterior pituitary is of the order of the maximum values of activity reported in either acute pancreatitis in the human or following pancreatic duct ligation in dogs.

There is a close parallel between the rise in serum amylase activity seen in these experiments and the increase in insulin sensitivity reported elsewhere following hypophysectomy (10). In rabbits the initial increase in insulin sensitivity is sometimes demonstrated at twenty-four hours after hypophysectomy; the maximum sensitivity is usually reached at forty-eight hours after operation. In the present experiments a similar

TABLE 2

Effect of serum concentration on glycogen loss of normal dog blood serum; average of three experiments

	SERUM CONCENTRATION					
	2.5 Per cent	5.0 Per cent	7.1 Per cent	10.0 Per cent	12.5 Per cent	15.0 Per cent
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Glycogen loss	9.7	14.2	16.2	16.5	16.9	17.4

time relation is shown between operation and the rise of serum amylase activity to maximum. It is probable, although not proven by these experiments, that it is the anterior lobe which exercises the control over serum amylase.

These experiments throw no light upon either the source or the function of serum amylase. It is not clear how the pituitary exercises its influence. Hayes and Salter have demonstrated a decrease in liver amylase activity in the hypophysectomized rat (11). Either the blood serum amylase activity does not vary directly with liver amylase activity or the conditions are different in the rat from the dog.

The influence of adrenin on serum amylase has received but little attention. Cohen reported a decrease in activity following adrenin but his dogs were all under an anesthetic. The nature of the anesthetic was not reported. In the unanesthetized dogs reported in this paper, adrenin, given in doses sufficient to give a moderate blood sugar rise, caused if anything a rise in serum amylase activity. Hypophysectomy did not alter significantly this response.

The significance of the experiments quoted in this paper in relation to acute pancreatitis and ligation of the pancreatic ducts is not clear. It

might be argued that part of the initial rise immediately following hypophysectomy could be due to a transient pancreatic degeneration following hypophysectomy. Petechial hemorrhages in the pancreas are commonly found during the first week following hypophysectomy. The sustained rise in serum amylase activity could not, however, be due to this cause. There are no later anatomical changes found in the pancreas following hypophysectomy suggesting a disorder of acinar cell function although there may be some degree of general atrophy of the gland as a whole.

CONCLUSIONS

Following hypophysectomy in the dog there is more than a twofold increase in activity of the blood serum amylase. This increased activity is sustained for as long as seventy-two days.

It is confirmed that foods, fasting, glucose, and insulin do not alter the serum amylase activity in the normal dog. Following hypophysectomy the high level of serum amylase activity was not significantly altered by food, glucose or spontaneous hypoglycemia.

A decrease in serum amylase activity following adrenin is not confirmed. The level of activity either rises slightly or is maintained both in the normal and hypophysectomized dog.

It is concluded that the anterior pituitary, presumably through its influence on carbohydrate metabolism, is a major factor in the control of blood serum amylase activity.

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METABOLISM AND BODY TEMPERATURE OF NORMAL AND ADRENALECTOMIZED RATS DURING EXPOSURE TO COLD

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The effect of cold environmental temperatures upon the basal metabolism of rats has been carefully investigated by Benedict and MacLeod (1929). They have shown that rats have a lower basal metabolism in summer than in winter and that previous exposure to cold tends to elevate the basal metabolism measured at 28°C. Mason (1934) noted a difference in basal metabolism of the same persons living in different climates. I have confirmed the stimulating effect of cold on the basal metabolism of rats (see Ring, 1936) and have been searching for an endocrine factor which might account for the long-continued elevation of metabolism which the cold produces. I was unable to show that the thyroid had any importance in this effect and decided to study next the adrenal cortex. Many workers have shown that an adrenalectomized rat will not maintain its body temperature in cold environments which normal animals can endure (see Houssay and Artundo, 1928; Belding and Wyman, 1926). Hartman, Brownell and Crosby (1931) made a study of metabolism in adrenalectomized rats during exposure to cold and state that the fall in body temperature is due to decreased heat production.

To investigate further the increased basal metabolism produced by cold, I have used two procedures. First, since completely adrenalectomized animals will not long survive in the cold, I have removed one gland completely and the medulla plus most of the cortex of the other. A large proportion of these animals survived temperatures of 5° to 7°C. for one week and their metabolisms were studied at 28°C. The second type of experiment consisted in measuring continuously the metabolism and body temperature of completely adrenalectomized rats during exposure to cold. This has given a better idea of how body temperature and metabolism fall.

METHOD. The apparatus used for the measurements of metabolism was similar to that described by Benedict (1930) except that one-quart preserving jars served as animal chambers and were immersed in a water bath. Spirometers were arranged to record graphically. Irregularities in their records indicated movements of the animals. Oxygen consump-

tion alone was determined. Respiratory quotients were assumed to be 0.72 (see Benedict, 1930). Occasionally a few open-circuit metabolism measurements were made and these substantiated the value used for respiratory quotient and checked the figures for metabolism determined with the closed circuit. In order to get reliable readings the animals were kept in the metabolism apparatus for at least 6 hours. Surface area was estimated by the formula devised by Lee (1929).

For the second type of experiment continuous measurements of body temperature as well as metabolism were desired. To determine body tem-

TABLE 1

Metabolism of normal rats

(Cal. per sq. meter per day, measured at 28°C.)

NUMBER	PRELIMINARY	AFTER LIVING AT 35°C. FOR ONE WEEK	4 DAYS LATER	AFTER LIVING AT 5–7°C. FOR ONE WEEK	2 DAYS LATER	FINAL
1	755	745	691	907	849	782?
2	733	730	672	953	767	756
3	764	709	727	869	848	756
4	696	669	678	910	767	727
5	732	638	705	824	801	708

TABLE 2

Metabolism of partially adrenalectomized rats

(Cal. per sq. meter per day, measured at 28°C.)

NUMBER	PRELIMINARY	AFTER LIVING AT 35°C. FOR ONE WEEK	4 DAYS LATER	AFTER LIVING AT 5–7°C. FOR ONE WEEK	2 DAYS LATER	FINAL
12	668	659*	683	1,010	700	791?
13	773	631*	877?	1,080	797	735
14	713*	694	711	857	686	668
15	541*	677	645	796		742
16	698*	660	663	828		723

* All of one adrenal and three-quarters of the other removed two days before this determination of metabolism.

perature thermocouples were placed in the colon. At first constantan-copper couples were used, following the method of Benedict and Parmenter (1929). Later these were replaced by constantan-iron couples and the temperatures recorded with a Micromax apparatus (Leeds and Northrup Company) Fine insulated constantan and iron or copper wires were soldered together and threaded through rubber tubing not more than 2 mm. in diameter for a length of 8 cm. The union was left protruding about 2 mm. At the other end of the fine tubing the wires were more heavily insulated with thicker rubber and were surrounded by a spiral

spring to a point where they left the animal chamber. This spring was very flexible and yet prevented the connections from being destroyed by the rats.

The wires inside the fine rubber tubing could be slipped through the rectum and far enough into the colon to set the junction near the liver. Since feces are often 8 mm. in diameter the insertion of a tube one quarter that size did not cause any extraordinary distention of the intestine. To hold the thermocouple in place, the projecting wires were attached to the tail with adhesive tape. The animals frequently did not appear disturbed when the thermocouples were being inserted, but often they seemed irritated for a while after the adhesive tape was applied to the tail. Usually they became quiet in about an hour, and thereafter the tests were started.

In some experiments I wished to give adrenaline without opening the animal chamber or in any way disturbing the animal. This was accomplished by passing into the colon a small rubber tube along with the thermocouple. The tube was connected with a syringe outside the animal chamber and could be used at any time during the experiment.

RESULTS. In tables 1 and 2 will be found the measurements of metabolism on normal and partially adrenalectomized animals before and after exposure to cold. These observations show that like the normal animals the partially adrenalectomized had an elevated metabolism as a result of living in the cold. It seems unlikely that the small amount of cortex remaining could have regrown sufficiently to account for this result.

Completely adrenalectomized rats placed in the metabolism chamber, which is surrounded by water kept at 20°C., will not survive more than a few hours. The moderate cooling due to this bath temperature is accentuated by the rapid ventilation which changes the air in the animal chamber twice a minute and of course carries with it much of the heat the animal produces. Most normal rats have no difficulty in maintaining their body temperature in this environment; only if a bath of 2 to 3°C. is used will their temperature fall about as fast as that of adrenalectomized rats. For comparison several normal animals have been studied at this low temperature.

The metabolism and body temperature of two adrenalectomized rats 1 and 2 weeks after adrenalectomy are shown in figure 1. It will be noted that the metabolism and the body temperature fall more rapidly with the lapse of time. In a large series of such rats it was found that the average fall of body temperature on the second day after the operation was 2° per hour; on the fifth day, 3°; on the ninth day, 5°. If only part of the adrenals was removed, no fall was noted unless there had been severe trauma. Cortin given to adrenalectomized animals keeps the heat production approximately normal (see Hartman, Brownell and Crosby, 1931).

In figure 1 it will be noted also that there is a fair parallelism between body temperature and metabolism. This is to be expected since the velocity of chemical reactions is affected by changes in temperature. By use of the Arrhenius equation normal and operated animals may be compared to see whether the failure of metabolism is due entirely to lowering of body temperature. No records were found in the literature expressing this relation in mammals. Therefore, the work was begun by placing

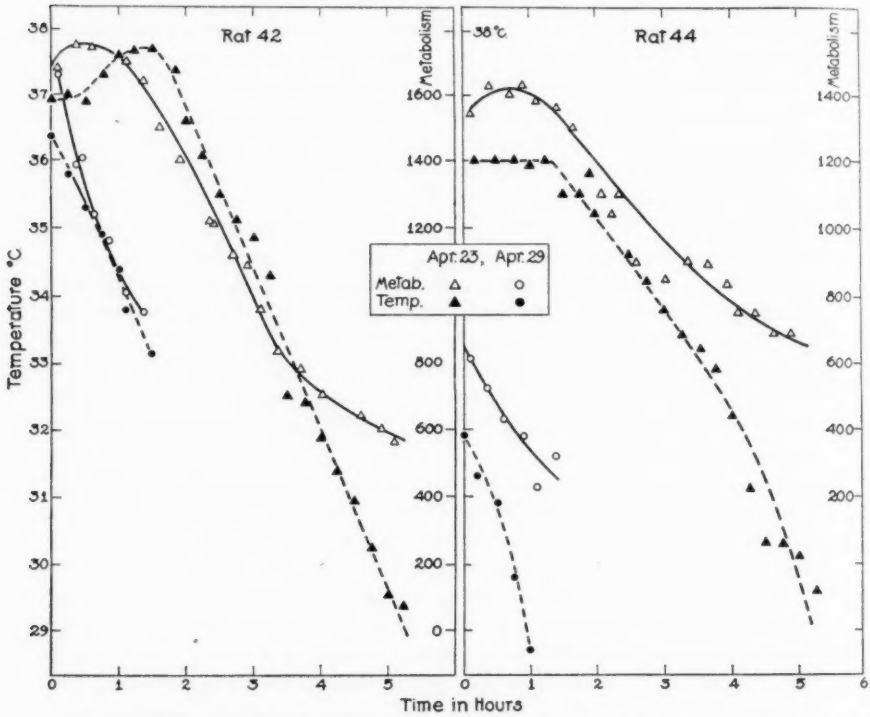

Fig. 1. Changes in metabolism (cal. per sq. m. per day) and body temperature of adrenalectomized rats during exposure to temperature of 20°C. Each rat adrenalectomized April 16.

normal rats in the metabolism apparatus with the water surrounding the chambers kept at 2 to 3°C. When the body temperature of these animals had fallen to about 27° the temperature of the bath was quickly raised to 30° and recovery was studied. I have discarded the results on all animals which died within 24 hours. The successful experiments have been plotted in figure 2 as the logarithm of the metabolism against the reciprocal of the absolute temperature of the body. In order to make the results come as

close together as possible in this and the succeeding chart, it was occasionally necessary to raise or lower by a constant amount the logarithms representing the metabolism of certain animals. This affects the intercept for such records but not the slopes of the line. It will be noted that the points for falling body temperature lie above those for rising temperature. In each animal observed, the metabolism in the warm bath lay well below

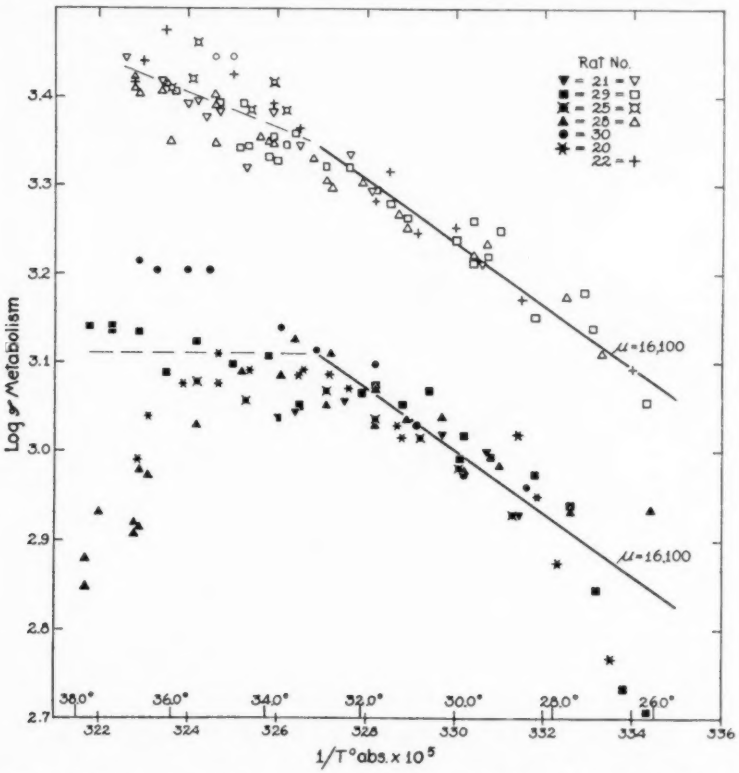

Fig. 2. Metabolism and body temperature of normal rats during exposure to temperature of 2-3°C. (open symbols) and after environmental temperature was raised to 30°C. (filled symbols).

that in the cold bath. The decreases in metabolism of six different animals at equal body temperatures were 38, 34, 29, 40, 34, and 31 per cent, respectively. Although body temperature was subnormal and a high metabolism was needed to raise this quickly, yet the metabolism fell. I suggest that part of the stimulus which elevated metabolism in the cold came from the skin; this was removed when the environment became

warm. It is important to observe that in spite of the difference in intercepts the slopes are the same for temperatures below 33°. According to the Arrhenius equation the μ value for the lines drawn is 16,100. A slope representing a value as high as 17,000 or as low as 15,500 might be selected. The figure chosen agrees with related observations summarized by Crozier (1924). Above 33° one can see no evidence that changes in metabolism

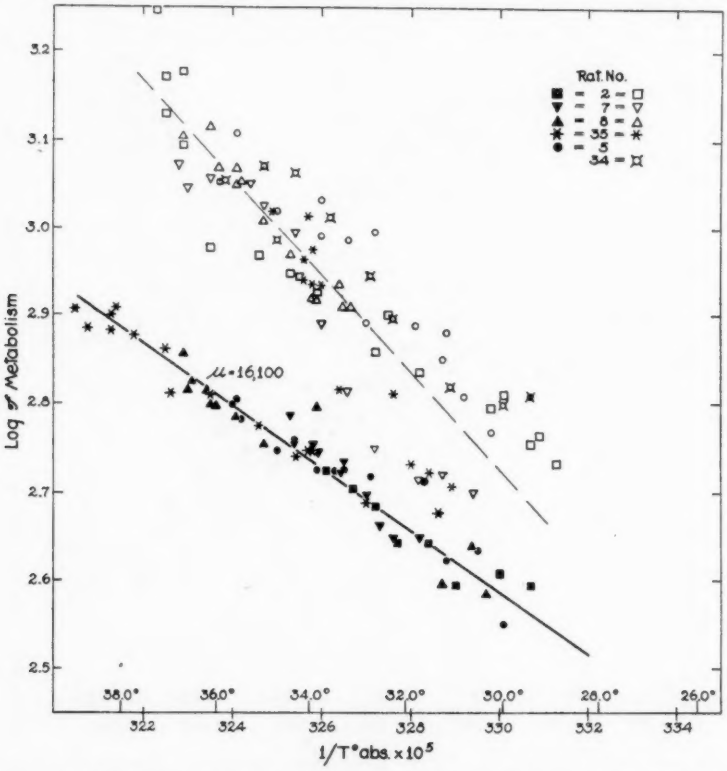

Fig. 3. Metabolism and body temperature of adrenalectomized rats during exposure to temperature of 20°C. (open symbols) and after environmental temperature was raised to 30°C. (filled symbols).

parallel those of body temperature. Other factors are entering either to oppose the falling body temperature or to retard the rising temperature.

In figure 3 similar records are given for adrenalectomized animals. In this case, the environmental temperature for studying falling metabolism and body temperature was 20° and not 2 to 3°. Furthermore, these animals, unlike normal ones, would seldom survive if the body temperature

dropped below 30°, so that the results were obtained over a narrower range than in the previous series. The warm bath used to follow rising body temperatures was kept at 30°. No regularity in the μ values for falling body temperature and metabolism exists for these animals, but the rising records are more consistent and give a μ value of 16,100—the same as that for normal animals. In figure 4 the results obtained during falling body temperature have been plotted at different levels. Frequently, these records show a regular slope for a time, followed by a sudden drop and then

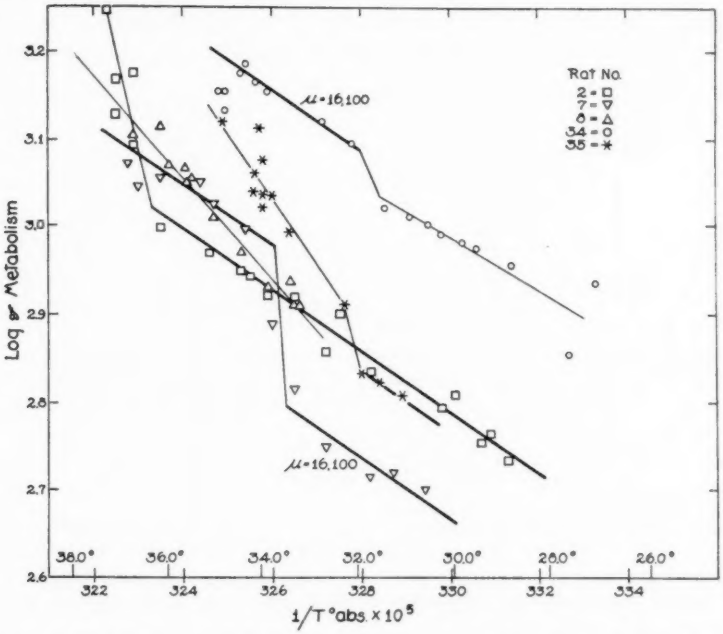

Fig. 4. Metabolism and body temperature of adrenalectomized rats during exposure to environmental temperature of 20°C. Records on individual rats plotted at different levels to show slopes.

another regular slope. Occasionally the slope is regular but much steeper than in normal animals. It looks as if the Arrhenius effect was complicated by another factor which is also tending to reduce the oxygen consumption. It is suggested that some of the muscles which shivered to keep up the heat production became fatigued and diminished their oxygen consumption. Ingle (1936) has shown that adrenalectomy in rats reduces their ability to do muscular work. In figure 3, it will be seen that the operated animals gave lower records with rising body temperature just

as did the normal rats. The decreases of metabolism in the warm bath at the low body temperatures amounted in different animals to 34, 33, 40, 31 and 26 per cent.

The inability to maintain heat production is not always due to lack of adequate supplies of energy. Operated animals, whether fed or fasting, showed the slowing of metabolism in about the same length of time. Giving glucose either by mouth or colon did not improve the metabolism. On the other hand, when adrenalin was given by colon, it very frequently elevated the metabolism and body temperature, showing that fuel was available but was not being used (see fig. 5). Adrenalin did not bring the body temperature and metabolism back to normal. It should be empha-

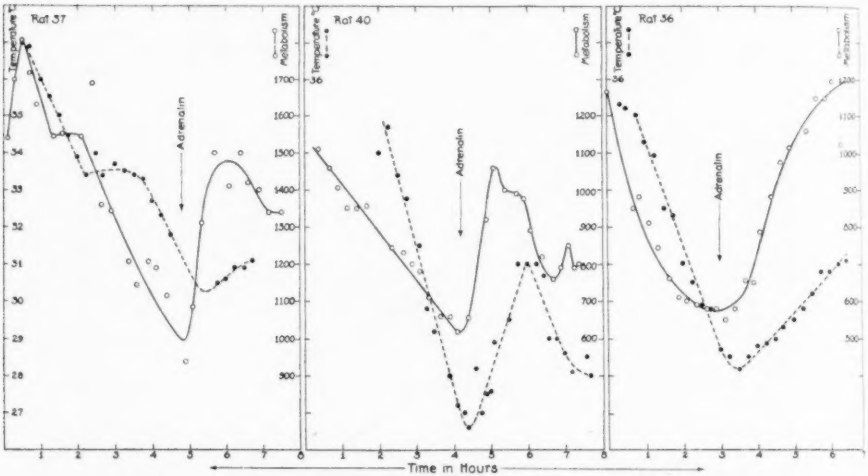

Fig. 5. Changes in metabolism and body temperature of adrenalectomized rats exposed to environmental temperature of 20°C. before and after receiving 0.5 cc. of adrenalin (1:1,000) per colon.

sized that animals with the adrenal medulla removed will maintain body temperature so that adrenine is not essential for normal heat production.

A lowering of metabolism and body temperature in normal animals may be produced by injecting 55 per cent glucose intraperitoneally (see fig. 6). According to Swingle, Parkins and Taylor (1936), this upsets the salt and water balance in a manner similar to that produced by adrenal insufficiency. As comparison of figures 1 and 6 shows, the course of events is not the same in the two conditions. One may also obtain lowered metabolism and body temperature by injecting a solution of KCl (see Truszkowski and Zwemer, 1936).

The difficulties in adrenalectomized animals are not corrected by giving

as drinking water the salt solution devised by Rubin and Krick (1933). All observations shown in figure 1 were made on such animals.

When adrenalectomized animals maintain their body temperature during the early periods of exposure to cold, the results may be compared with those obtained from normal animals kept at the same temperature. Such figures vary widely because differences in length of hair and subcutaneous

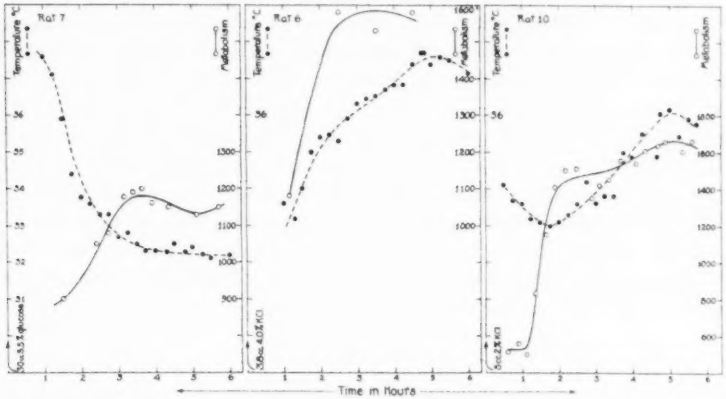

Fig. 6. Changes in metabolism and body temperature of normal rats exposed to environmental temperature of 20°C. after intraperitoneal injection of solution of glucose or potassium chloride.

TABLE 3

March 12, 1937. Rat 48 (adrenalectomized March 6)

TIME	R.Q.	CAL. PER SQ. METER PER DAY	BODY TEMPERATURE
a.m.			
10:00	0.75	1743	36.6
11:00	0.75	1696	36.2
11:20	0.74	1284	36.1
12:00	0.75	920	34.5
12:20	0.72	815	32.9
12:45	0.73	756	31.8

fat bring about different heat requirements. The average of one series of normal animals was 1420 cal., and of adrenalectomized rats was 1496 cal. The difference is probably not significant.

If the metabolism of the adrenalectomized rat is raised to the maximum, it never reaches 2,000 cal. per square meter per day, but the normal animal will commonly produce 2,500 cal. and more for several hours.

Some doubt may be cast on the validity of using oxygen consumption

alone as a measure of heat production when body temperature is falling. I have therefore examined the respiratory quotients in a few animals. The open circuit was used and analyses were made with apparatus devised by Carpenter (1933). The results are given in table 3 and show that changes in temperature did not affect this ratio.

Discussion. Webster, Pfiffner and Swingle (1932) have shown that injection of large amounts of cortical hormone into normal rats and rabbits does not affect their metabolism. Therefore, even if cold stimulates the adrenal cortex this gland could only elevate metabolism in the normal animal by releasing some other hormone than cortin. The observation that removing most of the adrenal cortex does not decrease the stimulating effect of cold is further evidence that the cortex probably has no importance in increasing oxidations. Complete adrenalectomy depresses many living processes which supposedly include oxidation secondarily. The more rapid fatigue of heat-producing parts under stress is one manifestation of the general derangement. It seems quite certain from the μ value obtained that the adrenal cortex has nothing to do with the "master" reaction for oxidation at the temperatures studied.

CONCLUSIONS

1. Normal rats show a critical thermal increment, or μ value of Arrhenius for oxygen consumption, of 16,100 (see fig. 2).

2. Adrenalectomized rats, while their body temperature is rising after exposure to cold, show the same μ value. During falling body temperature the μ value appears to be higher. This is probably due to simultaneous failure of some heat-producing mechanism which is independent of changes in body temperature. The true μ value appears to be unaffected by adrenalectomy (see figs. 3 and 4).

3. The intercept for the records during falling body temperature is higher than that during rising body temperature in both normal and adrenalectomized animals (see figs. 2 and 3).

4. Adrenalin will often stimulate the metabolism of adrenalectomized animals exposed to cold (see fig. 5).

5. Upset in salt balance produced by injection of solutions of KCl or glucose will diminish the expected heat production of normal rats during exposure to cold. Whether or not this derangement is similar to that which occurs in adrenalectomy was not determined. Attempts to improve the salt balance in operated animals by giving Rubin-Krick salt mixture did not correct the response to cold (see figs. 1 and 6).

6. Partially adrenalectomized rats maintain body temperature in the cold and have an elevated basal metabolism for some time after return to a warm environment (see table 2).

My thanks are due to Dr. H. Davis, who frequently offered valuable suggestions during the progress of this work.

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CARBOHYDRATE AND ELECTROLYTE CHANGES IN ADRENAL INSUFFICIENCY IN THE DOG[1]

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In most of our earlier reports on cortico-adrenal function we have considered chiefly the smaller laboratory animals, the cat, rabbit, rat, etc. That there are differences in the response of various animal species to adrenalectomy has, however, been pointed out in recent papers (Britton and Silvette, 1935, 1937). This is particularly the case in respect to the electrolyte changes (Silvette and Britton, 1936). Swingle and his colleagues have also concluded from their results (Parkins *et al.*, 1936) that in the dog the adrenal cortex is apparently not directly concerned with carbohydrate metabolism. Our preliminary work (Britton and Silvette, 1937) as well as recent experiments have indicated, however, that extensive carbohydrate changes occur after adrenal removal in the dog, as well as in all other types investigated. There appear to be important correlations, moreover, between the carbohydrate and electrolyte changes, and these have suggested the formulation of a broader concept of cortico-adrenal function which is set forth herein.

METHODS. Blood and tissue analyses have been made in several series of adrenalectomized dogs kept under different conditions, and comparison made with normal controls. Both male and female animals were used, the latter only when not in heat. In keeping with the observations of others, females adrenalectomized while in heat have been found to survive untreated sometimes for weeks without symptoms of insufficiency. To avoid serious shock a two-stage operation has been done in all cases, with operative intervals ranging from 2 weeks to 2 months. The cortico-adrenal extract used was the routine material prepared in our laboratory by a modified Swingle-Pfiffner process (Ehrenstein and Britton, 1937), and was practically adrenalin-free (less than 1 in 3,000,000, by blood-pressure assay).

Sodium was determined by the uranyl-zinc acetate method of Butler and Tuthill (1931); potassium by Kramer and Gittleman's (1926) gaso-

[1] Aided by a grant from the Division of Natural Sciences of the Rockefeller Foundation.

[2] E. R. Squibb and Sons Fellow in Physiology.

TABLE 1

Carbohydrate levels in adrenalectomized dogs showing symptoms of insufficiency

DOG NUMBER	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	CARDIAC GLYCOGEN
	mgm. per cent	*per cent*	*per cent*	*per cent*
1	66	0.19	0.39	0.42
2	70	0.26	0.61	0.15
3	40	0.28	0.23	
4	63	0.23	0.18	
5	57	0.34	0.30	
6	77	0.16		0.21
7	49	0.26	0.42	0.66
8	70	0.21	0.54	0.15
9	64	0.24	0.67	
10	58	0.20		
11	52	0.09	0.15	
12	75	0.15	0.48	0.10
13	70	0.14		0.15
14	73	0.16	0.47	
15	62	0.11	0.47	
16	66	0.23	0.49	

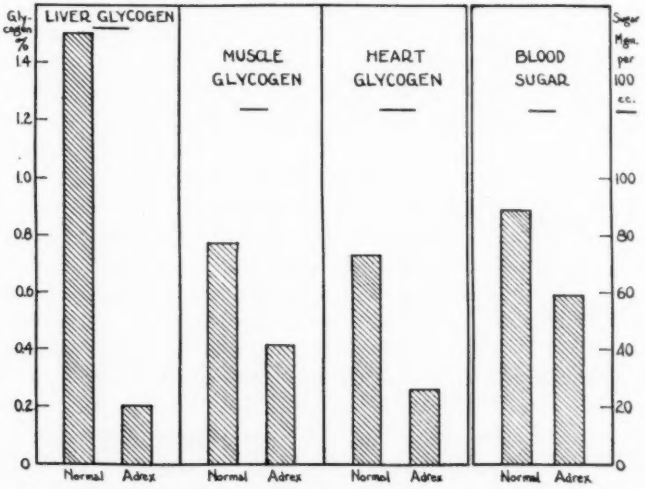

Fig. 1. Liver, muscle and heart glycogen and blood glucose levels in normal and adrenalectomized (adrex) dogs.

metric modification of Kramer and Tisdall's cobaltinitrite method (1921); chlorides by the method of Van Slyke and Sendroy (1923); urea by the manometric hypobromite method of Van Slyke (1929); sugar by Folin

TABLE 2

Changes in various blood constituents of adrenalectomized dogs following injection of cortico-adrenal extract

(Animals tested at various survival periods after adrenal removal; in some cases symptoms had appeared)

DOG NUMBER	DATE OF EXPERIMENT	TIME AFTER INJECTION	AMOUNT OF EXTRACT GIVEN	BLOOD SUGAR	BLOOD UREA	SERUM SODIUM	BLOOD CHLORIDES
		hours	*cc.*	*mgm. per cent*	*mgm. per cent*	*mE./l.*	*mE./l.*
1	March 9	0	20	74	278		71.1
		4		87	244		72.4
		13		95	150		72.7
2	April 21	0	30		361		68.2
		2	10	74	361		70.2
		17		123	312		70.2
	April 22*	0	30	107	287		73.9
		5		122	257		75.2
	April 23*	0	15	91	203		62.0
		15	30	117	124		71.9
		20		129	117		74.3
3	May 15	0	40	65	94		76.2
		24		84	71		68.2
4	June 5	0	60	69	171	136.6	
		64		94	120	141.1	
5	October 18	0	50	78	120	138.5	
		4		104		141.7	
		21		100		134.6	
		29		95		132.2	
	October 26	0	50	52		150.0	
		10		84		141.0	
6	October 27	0	50	53		125.4	
		24		87		121.9	
7	November 20	0	90	67	143	120.9	
		13		91	110	114.9	
	November 22	0	50		80	125.2	
		26			23	117.0	

* No insufficiency.

and Malmros' colorimetric ferricyanide method (1929); and glycogen by a modified Pflüger procedure (Silvette and Britton, 1932).

RESULTS. Untreated adrenalectomized dogs survived for various periods up to 21 days before symptoms appeared. They were generally utilized for tissue and blood samples when weakness had become well developed, or (sometimes) when the prostration stage had been reached. In nearly all cases, whether the survivals were long or short, the blood sugar levels were found to be much reduced. In the short-lived dogs (surviving only 1–2 days after adrenalectomy) the levels showed practically the same average as in the longer-surviving animals (3–21 days)—58 mgm. and 56 mgm. per cent respectively. The average in over 50 dogs was 59 mgm. This contrasts with an average of 89 mgm. in 10 normal dogs. The adrenalectomized levels were thus about 40 per cent below normal.

Liver glycogen levels in adrenalectomized dogs averaged 0.20 per cent in comparison with 1.50 per cent in normal animals, and muscle glycogen 0.41 per cent compared to a normal of 0.77 per cent (table 1). These represented decreases of over 85 per cent and 45 per cent respectively in adrenalectomized animals (fig. 1).

In several cases determinations of heart glycogen were made; samples were taken from the beating heart, and set up immediately in 30 per cent KOH. The values ranged from 0.10 to 0.66 per cent, and averaged 0.26 per cent in 7 adrenalectomized dogs, in comparison with an average normal value of 0.73 per cent (14 cases). In contrast, there were no reductions of cardiac glycogen observed in normal dogs after 4 days' fasting, and also none after severe hemorrhage.

The changes in serum sodium and serum (or blood) chlorides, and in serum potassium and blood urea, were essentially similar to those observed by other workers. When symptoms were well developed, in our series of adrenalectomized dogs, there were approximately 15 per cent decreases in both serum sodium and chloride, 100 per cent increase in serum K, and 500 per cent increase in blood urea.

When kept on small amounts of cortico-adrenal extract prepared in this laboratory, without any dietary precautions or salt administration (see Ehrenstein and Britton, 1937), normal blood conditions after adrenal removal were well maintained. Following the withdrawal of extract for several days, there occurred in all cases carbohydrate changes similar to those described above. A number of dogs were restored again and again by extract administration. Often, when severe weakness had set in, large doses were necessary to effect restoration. Such cases provided opportunity for determination of extract effects. Changes in various blood constituents were followed during the development of and recovery from insufficiency (table 2).

Serum sodium and blood chloride levels showed only slight, insignificant changes within the first 30 hours after extract administration to dogs with symptoms, although during this time recovery from insufficiency had occurred. The extract was made isotonic with sodium chloride, it should be stated, in many cases. Blood sugar changes, however, were uniformly upward, and blood urea shifts were at the same time uniformly downward. In 7 cases in which extract brought about restoration within 30 hours (the first signs of recovery usually having appeared within 1–2 hours), the blood sugar rose from an average of 66 mgm. to 95 mgm. per

TABLE 3

Blood chemical changes following sodium chloride solution injections in adrenalectomized dogs

DOG NUMBER	DATE	0.9 PER CENT NaCl SOLUTION INJECTED	BLOOD SUGAR	SERUM SODIUM	BLOOD CHLORIDES	REMARKS
		cc.	*mgm. per cent*	*mE/l.*	*mE/l.*	
10	April 26	400	94		74.1	Animal well; eating
	27	250	58		80.1	Refused food
	28					Weak
	29	600	38		85.1	Prostrated; death later in day
11	May 26	180	62		70.5	Refused food
	26	180	62		88.0	Animal weaker
12	October 23	600	90	122.9		Normal
	24	450	78	150.0		Weak
	25	250				Weaker
	26	400	52	150.2		Prostrated; death later in day
13	November 20	200		114.9		Animal weak
	21	200		125.2		Condition unchanged

cent—about 45 per cent. The blood urea, considering all cases, was consistently reduced by an average of 25 per cent from the pre-injection levels—from 202 mgm. to 145 mgm. per cent.

A series of adrenalectomized dogs which were given large quantities of normal saline were studied over periods of several days. In these the blood sugar levels were observed to decline, while the serum sodium or blood chlorides rose to normal values (table 3). Adrenal insufficiency nevertheless developed; two animals died within a few days, while others were saved by extract. Our lowest blood sugar and liver and muscle glycogen values have been observed in animals given saline over long periods.

The effects of adrenalin injection on blood sugar were observed in a few adrenalectomized dogs in the pre-symptom stages of insufficiency, or when slight lethargy appeared. No significant changes occurred: sometimes small rises in blood sugar and at other times reductions took place.

Discussion. In a recent paper, Parkins, Hays and Swingle (1936) conclude that the adrenal cortex is not directly concerned in the regulation of carbohydrate metabolism. Analysis of their data shows, however, that there occurred reductions in blood sugar from an average of 115 mgm. to 65 mgm. per cent in 8 cases within 26 hours after adrenal removal. In 28 other cases of adrenal insufficiency their average was 77 mgm., again a subnormal value. In contrast, in all other types of severe operation with rapid death, and in shock, etc., marked hyperglycemia has been shown to occur (Britton and Silvette, 1937). Large doses of adrenalin given to adrenalectomized dogs over periods of several hours by Parkins *et al.* failed to evoke hyperglycemia, and severe trauma was also ineffective. Their figures show blood sugar increases, however, on injecting cortico-adrenal extract, in 3 out of 4 cases.

Two adrenalectomized dogs, one male and one female, which were kept alive by Allers and Kendall (1937) on sodium salts for periods of 84 and 115 days respectively, showed low blood sugar values, and hypoglycemia occurred terminally in both cases. Although they do not consider the carbohydrate changes as highly significant, Allers and Kendall state that their adrenalectomized dogs sometimes showed severe hypoglycemia, and that in some cases this may be the cause of death.

In a classical review on the internal secretions published 25 years ago, Biedl (1913) concluded that the adrenals effect the formation of glycogen and the mobilization of sugar in the body. More recent reviews have emphasized the importance of the cortico-adrenal tissues in connection with the storage and utilization of carbohydrates (Britton, 1930; Laquer, 1934; Thaddea, 1936). Thaddea has emphasized that there occur profound losses in blood sugar (50–60 per cent) and liver and muscle glycogen 50–90 per cent) in adrenalectomized animals, including guinea pigs, rabbits and cats. Cortico-adrenal extract was shown to maintain or restore fairly normal values. The prime significance of the adrenal cortex in the regulation of glycogen in the tissues has also been commented on by Deuel (1937).

Our present studies in the dog supplement those made on the rat, guinea pig, rabbit, cat, marmot and opossum. In all cases very serious glucose and glycogen losses have been observed in adrenal insufficiency, involving deficits of more than 50 per cent of the total carbohydrate material in the body. In the rat and dog, it is true, the blood sugar levels are not often reduced to the shock level, and hypoglycemic convulsions are not as frequently observed as they are in the cat. In this connection it

should be noted that both the rat and dog usually take food within 12 hours of death from insufficiency. Thus their tendency to maintain the blood sugar above shock level is understood. Hepatic glycogen reserves are nevertheless almost completely exhausted in these as well as in other animals.

Two important considerations may be mentioned. Mann (1937) has pointed out that very rarely is the liver devoid of glycogen. This con-

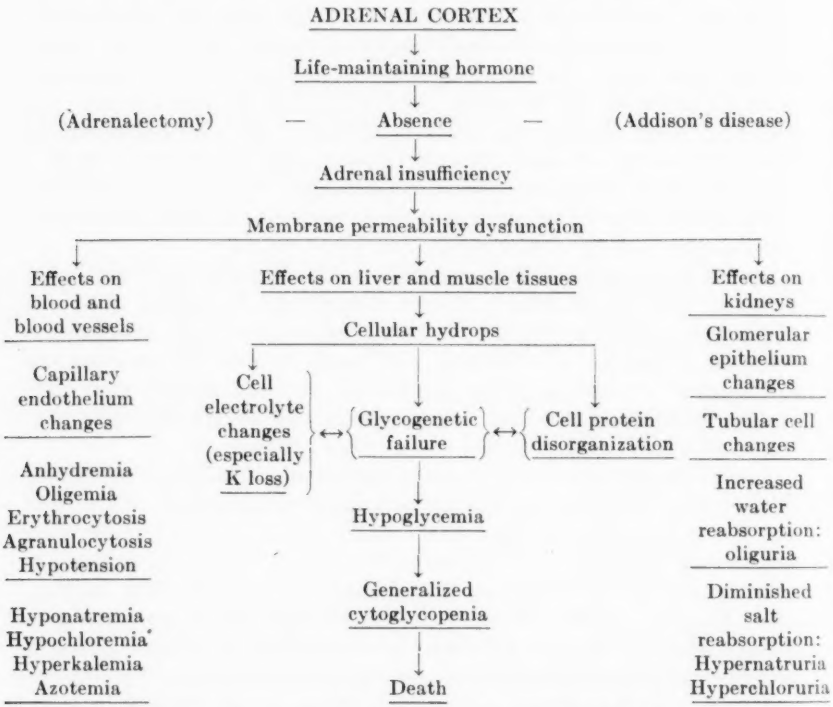

Fig. 2. Diagram illustrating the most generally recognized disturbances found in adrenal insufficiency and their probable order of occurrence.

dition is approximated in adrenal insufficiency rather frequently, however, in the experience of many workers (Britton, 1930; Thaddea, 1936). Again, Evans has shown (1934) that the heart (of rats) seldom suffers any notable reduction in its glycogen content—in cases of fasting, exhaustion by exercise, moderate anoxemia, electrical stimulation, and acid-base changes in the blood, no significant changes were observed. After adrenal removal, in contrast, we have observed that marked reductions in cardiac glycogen

occur. Interpreted in conjunction with the foregoing, these observations emphasize that the adrenal cortex is specifically concerned in regulating carbohydrate metabolism in the body.

The outstanding conditions observed in adrenal insufficiency are listed and correlated in figure 2. A mass of evidence which cannot be covered completely here has contributed to this scheme. In numerous papers we have discussed salt and water balance and carbohydrate metabolism, osmotic and permeability conditions in the adrenalectomized animal (Britton, 1930; Britton and Silvette, 1931–37). Important fundamental correlations between various constituents of plant tissues, shown in a recent review by Gregory (1937), may have a bearing on regulatory conditions in higher forms. Potassium and carbohydrate and protein metabolism are said to be very intimately related. It is found that potassium is highly necessary for maintaining the protoplasmic complex in cells, and that in its absence rapid proteolysis occurs (see Gregory). An extraordinary influence of potassium concentration on sugars leads to the conclusion that this substance is greatly concerned in the regulation of carbohydrate metabolism. Recent evidence from this laboratory confirms the foregoing (Silvette and Britton, 1937). Evans (1936) and Long and Lukens (1936) have related protein-carbohydrate conversion to cortico-adrenal function, while Verzár and Jeker (1936) re-implicate the adrenal cortex in lipoid metabolism. These and many related observations have been synthesized into a fairly consistent whole in the accompanying diagram. While questions may arise regarding the particular order of changes in adrenal insufficiency, the scheme given here indicates a highly probable progressive deterioration which is in agreement with practically all the scattered experimental evidence.

SUMMARY

There are pronounced carbohydrate deficiencies in adrenal insufficiency in the dog, similar to all other animal types investigated. In over 50 experiments the average blood sugar levels were reduced 40 per cent, although in many cases not to the shock level. Liver glycogen was reduced 85 per cent below normal—practically to exhaustion in the greater number of cases—heart glycogen about 75 per cent and muscle glycogen 45 per cent. In short survivals (up to 2 days) the blood sugar fell about the same as in longer survivals (2–21 days). These represented the most serious changes observed in adrenal insufficiency in the dog.

Cortico-adrenal extract restored normal blood sugar levels and markedly reduced the high blood urea values within 12 or 24 hours after injection in dogs with adrenal insufficiency. During the same period there were only insignificant changes in serum sodium or blood chloride levels.

Following the injection of large amounts of sodium chloride in dogs

with adrenal insufficiency, the levels of serum sodium or blood chlorides rose to normal, while the blood sugar concentration fell. Survivals were thus extended only a few days in such cases.

Adrenalin injections failed to raise the blood sugar significantly in adrenalectomized dogs.

Recent important work has shown that there is strong support for the belief that serious carbohydrate disturbances occur in the adrenalectomized dog.

A comprehensive scheme is presented which sets forth in diagrammatic and progressive order the outstanding changes which occur following removal of the adrenal glands. Permeability changes affecting especially the liver and muscle tissues are quite possibly responsible for initiating electrolyte and protein disturbances, and these are accompanied by (or cause, or depend on) the profound disorganization in carbohydrate metabolism which in most animals at least brings about death.

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INFLUENCE ON THE DURATION OF GESTATION ON THE INJECTION OF PREGNANCY URINE EXTRACT IN THE RAT BEFORE AND AFTER IMPLANTATION[1]

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The following experiments are reported as a contribution to the investigation of prolonged gestation in the albino rat. The attempt is made to determine the effect of a single injection of human pregnancy urine extract administered either before implantation or shortly afterwards.

The work was a direct outgrowth of experiments carried on in this laboratory (Hoopes, 1934) which showed that a single injection of human pregnancy urine extract (Antuitrin-S) given late in the gestation period of the rat invariably delayed parturition and resulted in the birth of postmature fetuses, as Synder (1934) had demonstrated in the rabbit. The next step was to determine the influence upon implantation and upon the birth mechanism, when injection was made early in the gestation period.

METHOD AND MATERIALS. Forty-one rats of Wistar stock were used, being maintained under standard conditions. Vaginal smears were made each morning from females in the mating cages and the day that spermatozoa were present was counted as day one of pregnancy. The placental sign was also observed, which provided the first definite evidence of pregnancy. The duration of gestation in this colony is 22 or 23 days, consequently, parturition after 23 days was considered delayed. With 2 exceptions, one intraperitoneal injection of 50 rat units (0.5 cc.) of Antuitrin-S, the Parke, Davis Company's preparation, was given to each animal. Seventeen rats, which will be referred to as belonging to group 1, were injected on days 3 to 6; and 23 rats of group 2 on days 7, 8, 9, or 10; and 1 rat on days 5, 7, and 11.

RESULTS. *Course of pregnancy.* Fourteen of the rats of group 1 injected on or before the day of implantation, bore litters at term. In 3, although the placental sign indicated that they were pregnant, gestation was interrupted about day 14, marked external bleeding occurring in 2 cases. There was one instance of delay, no. 1, injected on day 3. She bore normal young on day 30. These were killed and she was again pregnant 11 days later. The second litter was born at term, indicating that

[1] Aided by a grant from the Faculty Research Fund of Goucher College.

she had suffered no permanent injury. According to Teel (1926), implantation occurs on day 6, and Huber (1915), states that "during the 7th day after insemination, blastodermic vesicles become definitely oriented in a decidual crypt."

Fifteen rats injected 1 to 3 days later, when placental tissue had become a factor in the problem, with the exception of no. 27 showed a prolongation of from 2 to 3 days. Two animals injected on days 7 and 8 had litters at term, while in 4 cases parturition was delayed 12 to 24 hours.

State of the fetuses. The litters of the 20 rats in which parturition was delayed, with the exception of no. 1, group 1, noted above, all presented abnormalities; there were always some postmature fetuses, judged by the weight and the condition of the skin; the majority were dead at birth or when removed at autopsy. Occasionally there were 1 or 2 living young and these might be postmature in size but they did not live more than 3 or 4 hours. Only once was a living fetus born on day 26. Some fetuses when born had evidently been dead for many hours, judging from the discoloration, but evidence of recent death was more frequent than in the series reported by Hoopes and King (1935). In order to observe the conditions of the fetuses, 2 animals injected on day 10 were killed on day 25, at the beginning of vaginal bleeding and before loss of weight had begun. Eleven of the 17 fetuses in these 2 litters were sufficiently active to move when handled, 3 had died recently, 2 were small and degenerate and a third was immature and imbedded in a discolored clot of blood. Attention should be called to a borderline case, a rat was injected on day 7 which cast her litter on day 24. Of the 7 fetuses, 1 was born dead and 2 of those alive were a little larger than the average at birth. They would no doubt have survived if the mother had not been killed for autopsy.

Complications of parturition. Labor in the cases of delayed parturition might continue for several hours and dead fetuses were occasionally found in the uterus at autopsy, many hours after the expulsion of the first member of the litter. In a previous investigation it was found that parturition continued for from 2 to 6 days or might fail to occur, when rats were injected on days 19 or 20. Again, in the present series, sometimes only 15 or 20 minutes intervened between births. Only rarely did it take an hour or more for a fetus to emerge after its head appeared, although this frequently occurred in the earlier series.

It is of interest that rats of group 2 usually reached their maximum weight on day 24 and sometimes held it for 24 hours. Vaginal bleeding began on day 24 or 25. The blood at first fresh in color, became darker and then bleeding stopped entirely until the beginning of parturition which was accompanied by a discharge of fresh blood or of dark discolored blood, corresponding to the condition of the fetuses. In one instance fresh

blood was found in one horn of the uterus and discolored fluid in the other. The placentas were always discharged with the fetuses and were never found in the uterus at autopsy.

As in previous work, it was observed that rats which had undergone delayed parturition as a result of injection of human pregnancy urine extract, could later have litters at term. Several animals injected before implantation, as well as a few of those injected on day 10, afterwards had a normal pregnancy, indicating that the uterus was not permanently damaged. However, 2 animals of this series were seriously injured in the course of treatment. No. 2 was injected on day 3 and bore 3 living young on day 23. The uterus, at the end of parturition was inverted and hanging from the vagina. Two immature fetuses were found at autopsy. The vagina of no. 26, injected on day 10, was distended on day 19, and the wall of the uterus appeared at the vaginal orifice. The distention increased during the next 3 days, and she was killed on day 22. It was necessary to cut through the pubic bones in order to release the uterus. Two small living fetuses were pressed below the symphysis. There were, in addition 6 living fetuses, nearly a gram below the average weight, and 2 immature dead ones. Again, no. 27, injected on day 10, although she bore a normal litter at term, was unable to suckle her young and all were dead or in a moribund condition on the following day.

Condition of the ovaries. The ovaries of these rats, as those of the earlier series, contained many more luteinized follicles than there were fetuses. A primipara, injected on day 10, was killed on day 12, for comparison with an untreated primipara also 12 days pregnant. The first was carrying 5 embryos and the second 10, and yet the ovaries of the first weighed 64.8 mgm. per 100 grams of body weight while those of the second were 26.8 mgm. per 100 grams. Large bright red corpora lutea were present in the injected animal in addition to cream colored ones, which latter set corresponded to those of the control. The microscopic examination of an ovary of each of these rats confirmed the gross observations. In the experimental animal, large follicles in the early stages of luteinization were present, in addition to the corpora lutea of pregnancy, similar to those of the control.

One or two large follicles were invariably seen in the ovaries of animals which had undergone prolonged gestation and the masses of lutein tissue were, possibly, a paler yellow than those described above or than those of no. 26 killed on day 22. Large corpora lutea of two different stages of development were distinguished in serial sections of the ovaries of rats on day 26. These corpora lutea were similar to those described by Hoopes and King (1935).

Discussion. It is significant that in 13 of the 17 rats injected with 50 R. U. of Antuitrin-S before or on the day of implantation, pregnancy

was not interfered with, while the same amount given 1 or 2 days later, when placental tissue had begun to grow, usually delayed parturition and when given 4 days later, with one exception, profoundly and regularly influenced the onset and duration of parturition. It is possible, to be sure, that the induced lutein tissue does not remain active for more than 15 or 16 days and that follicles luteinized by injections on day 6, for example, are in the same physiological condition on day 22 or 23 as are the corpora lutea of pregnancy.

These experiments are of interest, when considered along with the reports of other workers, in which the effect of several injections was studied. Delayed implantation or interruption of pregnancy followed repeated injections of an anterior lobe extract given during the first 6 or 10 days of gestation; when it was continued to the 13th day, the birth mechanism as well as implantation was interfered with (Teel, 1926, and D'Amour et al., 1933). Pituitary implants made during the first 3 days of pregnancy resulted in failure of implantation (Engle and Mermod, 1928). Repeated early injections of human pregnancy urine extract, nearly always interfered with implantation or interrupted pregnancy (Hain, 1934). Wislocki and Goodman, 1934, failed to produce interference with pregnancy in the rabbit by early injections of Antuitrin-G (pituitary extract) or of Antuitrin-S, although excessive amounts of lutein tissue resulted.

Some mated animals, injected between days 2 and 10 are not included in this report because they did not show the placental sign. It is possible that pregnancy was interrupted before this time, but at least in the majority of cases, there was no interruption of pregnancy. The one instance in which an injection given before day 6 prolonged gestation, can be explained only as due to failure of implantation at the usual time, since the fetuses were born alive and in every respect normal. That the injection has a rather profound effect on the animal, is indicated by the 3 cases of interrupted pregnancy in group 1 and the 1 case in each group noted above.

The results of these experiments indicate that the duration of pregnancy is related to changes in the ovaries and that emptying of the uterus is under hormonal control. They show further that events in the first half of pregnancy may influence its termination. Living fetuses are born at term, if injection is made before implantation, but normal pregnancy rarely occurs if injection is made after implantation.

SUMMARY

1. The effect on the course of pregnancy and on parturition was observed in 41 pregnant rats injected during the first half of gestation with one standardized dose of human pregnancy urine extract.

2. Injections made on or before the day of implantation, usually do not interfere with gestation and normal fetuses are born at term.

3. Injections made after implantation, within a period of 4 days, result in prolongation of pregnancy and the birth of postmature fetuses which may be alive.

4. The results show that parturition in the rat is under hormonal control which may be experimentally modified after implantation.

I wish to acknowledge my indebtedness to Dr. Franklin F. Snyder for valuable criticisms of this paper.

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THE RELATION OF THE ADRENAL CORTEX TO CARBOHYDRATE METABOLISM

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Animals suffering from adrenal cortical insufficiency sometimes manifest hypoglycemia as was first demonstrated in 1908 by Bierry and Malloizel (1) and frequently confirmed by subsequent observers (8). This tendency to hypoglycemia is most frequently observed in animals maintained alive for some time following adrenalectomy with an inadequate replacement therapy. Besides hypoglycemia as evidence of a relation of the adrenal cortex to carbohydrate metabolism, there is a diminution in the glycogen content of the liver and muscles, as was first demonstrated by Porges (20), and the mobilization of glycogen from glucose or from *d*-lactic acid is inordinately delayed (3, 13).

Except for the above mentioned well-established facts, other studies are conflicting and in part based on uncertain data. Some authors attribute to the adrenal cortical hormone an ability to induce hyperglycemic blood levels in normal animals. Others believe that the carbohydrate disturbances observed in hypophyseal insufficiency are mediated through the adrenal cortex. It has been suggested that the adrenal cortex may elaborate two hormones one of which is an important factor in regulating the normal carbohydrate levels of the organism. The experiments presented in the present paper bear upon various aspects of carbohydrate metabolism as they are affected by the adrenal cortex.

The effect of adrenal cortical extracts on the blood sugar of normal animals. Were the adrenal cortex as intimately involved in the regulation of normal carbohydrate metabolism as are, for example, the pancreas and pituitary body, one would anticipate that adrenal cortical extracts would produce a demonstrable change in the glucose level of the blood of normal animals. Zwemer, Britton, Riddle and their collaborators (2, 21, 25) found that extracts of the adrenal cortex induced hyperglycemia in normal animals. Numerous other investigators (8, 11, 24), on the other hand, have failed to observe an effect of cortical extracts on the normal blood sugar. The blood sugar level is a notoriously labile function, many substances when injected being capable of inducing hyperglycemia. Moreover, most

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

available cortical extracts are contaminated by numerous impurities (acetyl choline, epinephrine, phenolic derivatives, *etc.*) which induce hyperglycemia. Hence, the demonstration of hyperglycemia following the use of adrenal extracts is of significance only when control experiments with inactivated extracts (free of hormone but still containing the contaminants) are shown to be devoid of any hyperglycemic activity. Such control experiments have heretofore not been reported.

TABLE 1

The effect of the intraperitoneal injection of 1 cc. of adrenal cortical extracts on the blood sugar level of normal fasted rats

The values recorded are the average of single determinations on 6 different animals.

EXPERIMENT NUMBER	EXTRACT INJECTED	CORTICAL HORMONE CONTENT OF INJECTED FLUID	BLOOD SUGAR	
			Before injection	After injection*
		rat units	*mgm. per cent*	*mgm. per cent*
1	Author's extract	20	110	108
2	Author's extract concentrated and purified	50	105	107
3	Commercial extract 1	10	101	105
4	Commercial extract 2	8	110	126
5	Commercial extract 2 (purified)	7	100	105
6	Contaminants from commercial extract 2	1	99	133
7	Inactive liquors	0	102	122

* Average values of determinations made at ½ hour, 1 hour or 3 hours after the injection of the extracts. For the sake of brevity only average values are recorded. The variations of individual determinations did not exceed the unavoidable experimental fluctuations.

In table 1 are recorded a series of experiments on young adult rats injected with various types of extracts derived from adrenal glands. Blood sugar determinations were made on 0.1 cc. samples of blood (obtained by cardiac puncture on the unanesthetized animal) by the method of Hagedorn and Jensen (10). Groups of 6 rats were used in each experiment, the average result being recorded in the table. In each case, one cubic centimeter of an extract, the hormone content of which had been previously determined by the rat assay method (8), was injected intraperitoneally. The potency of the extracts expressed in rat units, as defined elsewhere (8), is given in column 3 of the table.

The injection of the most active extracts used in the present study (expts. no. 1 and 2) failed to elicit any hyperglycemic action. The extract

of experiment 1 was prepared[2] by the method of Grollman and Firor (9); the extract of experiment 2 was a highly purified concentrate, prepared as described elsewhere (8). The extracts of experiments 3 and 4 were commercial extracts of essentially equal potency. One of these extracts (expt. 3) did not elicit any hyperglycemic reaction, while the other (expt. 4) did. To determine the agent responsible for this hyperglycemic reaction, this extract was fractionated to separate the agent responsible for the hyperglycemic effects from the life-maintaining principle. This separation was brought about by the following procedure: The original aqueous extract was repeatedly extracted with ethyl acetate until all the life-maintaining hormone had been removed. Much inactive material (inactive as regards its ability to maintain life in the adrenalectomized animal) remained in the aqueous residue. The ethyl acetate extract was reduced to dryness *in vacuo* at a low temperature and extracted with cold acetone. The acetone solution was then fractionally precipitated with freshly distilled petroleum ether (boiling point, 30° to 50°). The active fractions were combined and the whole process was repeated. All the inactive fractions (as tested on adrenalectomized rats) were then dissolved in ethyl alcohol, combined with the original inactive aqueous residue and the combined extract reduced *in vacuo* to remove the alcohol and bring the final solution to its original volume. The active fractions, in turn, were combined and transferred in a similar manner to an aqueous solution equal in volume to the original extract. In this way it was possible to obtain two extracts. One of these contained less than 8 per cent of the activity of the original extract as tested by the rat assay method (8) and retained over 85 per cent of the total solid content of the original extract. The second was equal in potency to the original extract, as tested on the adrenalectomized rat, and was free of most of the extraneous solids present in the original extract. This purified extract (expt. 5) failed to elicit hyperglycemia. The products removed by the process of purification, on the other hand, induced the same degree of hyperglycemia as did the original extract (expt. 6).

It is thus evident that the hormone which maintains life in the adrenalectomized animal does not induce hyperglycemia. This is also demonstrated in experiment 7 in which the residue remaining after removing the hormone with charcoal (8) was injected.

The experiments of table 1 demonstrate the absence of any hyperglycemic effect in the normal fasted animal. In order to preclude the possibility of any delayed effects, a second series of determinations were made on normal dogs and rats in which the blood sugar level was followed at intervals up to 18 hours after the first injection. The results of these

[2] I am indebted to Sharp and Dohme and Mr. Robert L. Fox for their generous supplies of adrenal glands.

experiments are reproduced in table 2. The dogs had been fasted for 15 hours previous to the injections but were given their usual daily ration after the blood collection made 3 hours after the first injection. The rats were allowed free access to their usual food at all times. As is obvious from the table, even with the repeated injections of relatively large doses of hormone and with access to food, no demonstrable immediate or delayed hyperglycemia was invoked.

The effects of the cortical hormone on the carbohydrate metabolism of adrenalectomized animals. The injection of adrenal cortical extracts, as shown in the preceding section, is without effect on the carbohydrate

TABLE 2

The effect of the adrenal cortical extract on the blood sugar level of the normal animal

Five cubic centimeters of an extract containing 20 rat units per cubic centimeter were injected into each dog intravenously; 0.5 cc. was injected into each rat intraperitoneally. Three injections of the above mentioned doses were given each animal at hourly intervals.

ANIMAL SPECIES	SEX	WEIGHT	BLOOD SUGAR					
			Before injection	Time after 1st injection				
				15 minutes	30 minutes	1 hour	3 hours	18 hours
		kilos	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Dog	♂	5.1	101	105	110	108	98	102
	♀	4.7	110	100	114	111	101	112
Rat	♂	0.2	112	120	126	120	118	117
	♀	0.2	125	130	133	127	122	126

levels of normal animals. The question arises as to the ability of these extracts to maintain the normal carbohydrate levels of adrenalectomized animals. Although adrenal extracts have been shown to be capable of supporting adrenalectomized animals in an apparently normal state of health, more detailed studies of various factors involved in carbohydrate metabolism have revealed an inability of these extracts to replace completely the function of the adrenals in the adrenalectomized animal. Thus Long and Lukens (16) have found that the injection of a commercial extract of adrenal cortical hormone failed to restore the diabetic state of pancreatectomized cats after adrenalectomy. Evans (5) was unable to alter the sugar and nitrogen excretion of adrenalectomized-phloridzinized rats or to raise the glycogen of animals exposed to low atmospheric pressure by the injection of adrenal cortical extract. Buell, Anderson, and Strauss (3) noted that adrenalectomized rats maintained in good condition by the oral administration of a charcoal adsorbate of cortical hormone made

excellent use of ingested *d*-lactic acid. These animals manifested, however, deficient stores of carbohydrate as compared to normals. Fry (6) was unable to elicit the ketogenic effects of anterior pituitary extracts in adrenalectomized animals maintained on adrenal cortical extracts.

All of the above-mentioned authors assumed that the amounts of hormone administered to their animals were adequate. They, therefore, concluded that the adrenal cortex probably elaborated a principle (distinct from the hormone which maintains life in the adrenalectomized animal) the absence of which from their extracts was responsible for the deficiencies noted.

TABLE 3

A comparison of the carbohydrate levels of normal and treated adrenalectomized rats maintained on various doses of adrenal cortical hormone for seven days and fasted for six hours

EXPERIMENT NUMBER	THERAPY ADMINISTERED DAILY	DAILY DOSAGE	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN
		rat units	*mgm. per cent*	*gms. per cent*	*gms. per cent*
1	0.5 cc. commercial extract injected	3	95 ±5	1.0 ±0.2	0.46 ±0.02
2	0.2 gram charcoal adsorbate orally	3	100 ±8	1.4 ±0.3	0.48 ±0.01
3	0.3 gram charcoal adsorbate orally	5	110 ±4	1.8 ±0.3	0.53 ±0.04
4	0.05 cc. purified extract, injected	5	97 ±4	1.5 ±0.2	0.50 ±0.02
5	0.05 cc. purified extract, orally	5	112 ±6	1.7 ±0.3	0.55 ±0.03
6	0.05 mgm. crystalline preparation, orally	5	108 ±3	1.8 ±0.3	0.51 ±0.02
*	Controls (unoperated)		111 ±5	1.7 ±0.3	0.53 ±0.01

* For the sake of brevity, the results obtained in all control animals of all the experiments have been averaged in the single "Control" series.

In order to test the capacity of adrenal cortical preparations to maintain normal carbohydrate levels in adrenalectomized animals, the experiments of table 3 were performed. Groups of six adult rats of the same age, sex, and weight were used for each experiment. Three rats were retained as controls; the others were doubly adrenalectomized, as described elsewhere (8). All the animals had constant access to the standard Steenbock-McCollum diet on which they had been maintained for a week preceding the experiment. The operated animals were treated with varying amounts of hormone (column 3) administered in different ways (column 2) for a period of one week, at which time untreated control animals manifest deficiencies in the level of their carbohydrate stores. Glycogen determinations were made by the method of Good, Kramer, and Somogyi (7).

As shown in experiments 1 and 2, the administration of 3 rat units daily did not suffice to maintain normal carbohydrate levels, although this

amount of hormone maintains adrenalectomized adult rats in apparently good condition (8). The maintenance of normal carbohydrate levels in the adrenalectomized animal is apparently a more sensitive indicator of adequate replacement therapy than the maintenance of life and body weight. The administration of the hormone orally admixed with the animal's food resulted in better replacement (expt. 5) than when the same extract was administered in two equally divided daily intraperitoneal injections (expt. 4).

It has been suggested that the adrenal cortex may elaborate two hormones one of which maintains life in the adrenalectomized animal while the other is concerned with the conversion of protein into carbohydrate and is essential for the maintenance of normal carbohydrate levels. Experiment 6 of table 3 was carried out to test this hypothesis. In this experiment a crystalline preparation[3] of the hormone was administered in minute doses. Normal carbohydrate levels were attained by the use of this compound. Hence it can not be argued that the relatively large doses administered in the other experiments were required because of the necessity of administering two principles, one of which was present in only small amounts.

The results of table 3 indicate the reasons for the failure of previous observers to obtain adequate carbohydrate levels or adequate replacement of adrenal function by the use of cortical extracts. The relatively small amount of hormone present in most available adrenal cortical extracts is not generally appreciated. Samples of all the available domestic[4] and one foreign preparation have been assayed by the present author using the technique described elsewhere (8). The most potent of the commercially available extracts contained from 6 to 10 rat units per cubic centimeter. Some manifested no demonstrable activity while others contained only 1 or 2 rat units per cubic centimeter. Nor is there any discrepancy between the requirement of the rat and larger mammals when one considers their respective surface areas (*cf.* table 2, page 243, reference 8).

It is thus probable that the failure by earlier workers to obtain complete replacement therapy, as indicated by carbohydrate deficiencies, in adrenalectomized animals was due to the use of inadequate amounts of hormone. The apparent well-being of an adrenalectomized animal maintained on a given therapy is no proof of the adequacy of this therapy from a physiological standpoint. To obtain complete replacement therapy it is necessary, as demonstrated in table 3, to administer an amount of ex-

[3] The identifying properties of this crystalline preparation have been described elsewhere (Cold Spring Harbor Symposia on Quantitative Biology, volume 5, p. 313, 1937). The crystals as obtained from ethyl acetate melt at 182°–3C.

[4] Dr. David Klein and Dr. George F. Cartland kindly supplied two of the commercial extracts.

tract several times as great as that which is required for maintaining life. Parenteral injection of a given dose is less efficacious in the rat than oral administration, probably because the rat's habits of feeding permit the exhibition of the hormone at a continuous rate. Only by the use of highly purified concentrates of the adrenal extracts which are available at present could one hope to secure an adequate therapy in larger mammals.

The effect of the adrenal cortical hormone in the adrenalectomized-depancreatized animal. The experiments cited in the preceding section demonstrate the ability of a single principle (the life-maintaining hormone) to maintain normal carbohydrate levels in adrenalectomized animals. In view of the experiments of Long and his co-workers (15, 16), it was deemed necessary to test the adequacy of the cortical hormone as a replacement therapy in the adrenalectomized-depancreatized animal. If the above-cited proposition be true, it should be possible to maintain the hyper-

TABLE 4

The effect of administering 5 units daily (orally) of the adrenal cortical hormone on the blood sugar and glycosuria of depancreatized-adrenalectomized rats

The results are averages of single determinations on 6 animals

	Before adrenalectomy	Five days after adrenalectomy
Blood sugar in milligrams per cent	195 ±12	197 ±10
Glycosuria in grams per rat per day	1.2 ±0.3	1.0 ±0.2

glycemia, glycosuria, and ketosis of the depancreatized animal after adrenalectomy at the level observed before removal of the adrenals. This has been attained, as described in the following experiments.

Eighty-six rats, one month of age, were partially depancreatized by the method of Shapiro and Pincus (23). As shown by these authors and by Long (15), such animals in the course of two weeks sometimes develop high blood-sugar levels and subsequently manifest glycosuria and ketosis. Two weeks following partial pancreatectomy a group of 12 of the animals whose blood sugar on successive days had averaged 195 ± 12 were bilaterally adrenalectomized. Half of the animals were then treated with 5 rat units daily of adrenal cortical hormone administered orally by admixture with their food. On the fifth day, as shown in table 4, the high blood-sugar levels found previous to the adrenalectomy were retained. The control, untreated *adrenalectomized-partially depancreatized* animals, had attained normal blood sugar levels on the fifth day following adrenalectomy, as has previously been found by Long (15).

The remainder of the partially depancreatized animals were allowed to grow to adult size. Twelve of these animals which showed a urinary

output of 1.2 ± 0.3 gram of reducing substances per rat per day were adrenalectomized and treated as in the first series. As shown in table 4, this glycosuria was maintained five days after adrenalectomy while the control untreated group showed only an insignificant degree of glycosuria at this time.

Ketosis. The formation of ketone bodies has been considered by several authors as an aspect of metabolism in which the adrenals are involved. Adrenalectomy markedly reduces the ketonuria observed in a number of metabolic disturbances; *e.g.*, after pancreatectomy (16), during fasting (18), after phloridzin administration (5), or after the injection of pituitary extract (6, 15, 18). This reduction in ketonuria induced by adrenal cortical insufficiency may be looked upon as analogous to the failure of the adrenalectomized animal to react to many other forms of stimulation; *e.g.*, to the hyperthermic effects of pyrexic agents (8). It is unnecessary to infer that the adrenal is normally the prime agent responsible for the formation of ketone bodies although its hormone may be essential for the normal production of these bodies by other organs.

The most cogent argument for the view that the adrenal cortex is normally involved in the production of the ketone bodies is based on the reputed ketogenic effects of adrenal extracts (17). A repetition of the experiments upon which this view is based has failed to substantiate its validity. The particular extract with which MacKay and Barnes (17) succeeded in inducing ketosis in normal fasted rats manifests, as Long (14) has shown, the reactions of the ketone bodies. The injection of relatively large doses of extract (2 to 5 cc.), as were used by MacKay and Barnes (17), might be expected to result in the appearance of these substances unchanged in the urine. Moreover, the impurities and preservatives (sulfur dioxide, phenol, alcohol, *etc.*) present in commercial extracts might also induce ketogenesis in the fasted rat.

To determine whether or not the ketogenic effects are induced by the hormone of the adrenal cortex, the ketone excretion of fasted rats was determined under the influence of a highly purified adrenal cortical extract. The methods employed were similar to those used by MacKay and Barnes (18). The results are reproduced in table 5. To avoid the marked individual fluctuations, groups of 12 animals were used in each experiment. Six of each group were injected with 1 cc. of a 0.9 per cent saline (as controls) while the others were each injected with an equal volume of a purified extract containing 50 rat units per cc. This amount of hormone should suffice to elicit any possible ketogenic action. It is equivalent in hormone content to the injection of 5 cc. of the most potent available commercial extracts.

The results reproduced in table 5 clearly show the absence of any ketogenic effect by the adrenal cortical hormone.

The relation of the adrenals to the carbohydrate deficiency in hypophyseal deficiency. Hypophyseal deficiency is generally known to be associated with a tendency to hypoglycemia and loss of the normal carbohydrate stores of the liver and muscles. When well fed, the hypophysectomized animal retains its carbohydrate stores at a normal level; when fasted, a rapid loss of liver glycogen occurs followed by hypoglycemia and a loss of muscle glycogen.[5] The hypophysis is apparently an organ essential for the maintenance of body glycogen stores and blood sugar during fasting (12, 19, 22).

In view of the changes occurring in the adrenal, it has been suggested that the carbohydrate deficiencies observed after hypophysectomy are due to a secondarily induced adrenal cortical insufficiency. Such a conclusion is open, however, to several objections. In the first place, no proof has been adduced that the adrenal after hypophysectomy is actually functionally deficient. The obvious reduction in the size of the adrenal

TABLE 5

The effect of the adrenal cortical hormone on the excretion of ketone bodies of normal fasted rats

Each experiment represents the average result obtained on a group of 12 animals during the second day of fasting.

SEX	INITIAL WEIGHT	KETONE BODIES IN MILLIGRAMS PER RAT PER DAY	
		Controls	Injected
	grams		
♂	200 ±5	2.5	2.7
♀	220 ±5	11.5	9.6

observed after hypophysectomy may be interpreted as a mere reflection of a decreased general metabolic activity of the organism (8). Moreover, the disturbance in carbohydrate metabolism which follows hypophysectomy is demonstrable, as Russell and Bennett (22) have shown, within 24 hours after the ablation of the pituitary, whereas carbohydrate disturbances in the mammal may not appear for many days following adrenalectomy (8, 24).

One can easily demonstrate whether or not the adrenals are responsible for the carbohydrate deficiencies of hypophyseal insufficiency by noting

[5] The diminutions in carbohydrate levels noted by Corey and Britton (4) in hypophysectomized rats following adrenalectomy are attributable to the diminished food intake observed in such doubly operated animals. The elevated blood sugars observed by the same authors in hypophysectomized rats are probably due to operative lesions of the tuber cinereum or hypothalamic excitation. Evidence for the extra-adrenal origin of the carbohydrate deficiencies noted in hypophysectomized rats has also been adduced by Samuels, Schott and Ball (This Journal **120:** 659, 1937) and by Bennett (Endocrinol. **22:** 193, 1938).

the effects of the administration of the adrenal cortical hormone on these deficiencies. The results of such experiments are cited in table 6. Young adult male rats were hypophysectomized by the parapharyngeal route. They were treated post-operatively for five days with 5 units of adrenal cortical hormone administered orally with their daily ration. On the sixth day they were fasted for 10 hours. During this period they received an intraperitoneal injection of 3 rat units of adrenal cortical hormone every third hour. The rats were then anesthetized and their blood and tissues analyzed for their carbohydrate content. The data for the unoperated controls (rats 1, 2, and 3) demonstrate the relatively slight effects of fasting on the carbohydrate stores of normal unoperated rats. The reduced values for these functions in the hypophysectomized treated rats (nos. 7, 8, and 9) differ little from those of their untreated controls. The

TABLE 6

The effect of administering 5 rat units of adrenal cortical hormone per rat per day on the carbohydrate reserves of hypophysectomized rats fasted for 10 hours

RAT NUMBER		BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN
		mgm. per cent	*grams per cent*	*grams per cent*
1	Unoperated Controls	104	1.2	0.50
2		107	1.0	0.52
3		101	1.4	0.51
4	Hypophysectomized Controls	61	0.04	0.40
5		72	0.13	0.43
6		57	0.06	0.38
7	Hypophysectomized Treated	64	0.08	0.44
8		76	0.10	0.39
9		50	0.07	0.37

administration of a relatively large dose of hormone would certainly ensure the absence of any adrenal cortical insufficiency in the treated animals. We must thus conclude on the basis of the results of table 6 that the carbohydrate deficiencies of hypophyseal insufficiency (at least as observed in its early stages) are not a result of a secondarily induced adrenal cortical insufficiency.

Discussion. It has been demonstrated in the present paper that the administration of the hormone of the adrenal cortex in large doses to normal animals fails to raise their normal carbohydrate levels to abnormally high values. Cortical extracts are capable, however, of maintaining normal carbohydrate levels in the adrenalectomized animal. The question arises: What is responsible for the deficiencies in carbohydrate metabolism observed in adrenal insufficiency? Several possibilities suggest themselves.

Adrenal insufficiency is manifested by demonstrable pathological deficiencies in the liver and muscles (8). These secondary changes induced by adrenal insufficiency would obviously account for such disorders in carbohydrate metabolism as have been proven to occur without assuming any more fundamental and direct control of metabolism by the normal gland. A second possibility is that the observed deficiencies are due to hypophyseal insufficiency. The probability of hypophyseal dysfunction in chronic adrenal insufficiency has been demonstrated (8) and it is not impossible that this dysfunction may manifest itself at an early stage of adrenal insufficiency. This view would be in accord with the demonstrated diabetogenic effects of pituitary extracts in adrenalectomized animals (12).

The results of the present work may appear to be in contradiction to the clinical observations of "diabetes" in the adreno-genital syndrome if one considers this disease as resulting from hyperfunction of the adrenal cortex without a concomitant over-activity of the hypophysis. The adreno-genital syndrome can not, however, be attributed to an overproduction of the life-maintaining hormone. Its manifestations are due to the elaboration of androgenic substances by a pathologically active tissue which either itself or through its effects on the hypophysis could induce the observed diabetogenic effects (8).

SUMMARY

1. Adrenal cortical extracts, potent as regards their life-maintaining capacity in the adrenalectomized animal, do not induce hyperglycemic blood-levels in normal animals.

2. The life-maintaining hormone of the adrenal cortex when administered to adrenalectomized or adrenalectomized-depancreatized animals maintains carbohydrate levels at their normal levels. Insofar as carbohydrate metabolism is concerned it is unnecessary to assume that the adrenal elaborates a second hormone distinct from the life-maintaining principle.

3. Purified potent extracts of the adrenal cortex do not stimulate ketogenesis in the normal fasted rat.

4. The adrenal cortex is not responsible for the deficiencies in carbohydrate metabolism observed after hypophysectomy.

5. The deficiencies in carbohydrate levels observed in adrenal insufficiency are probably attributable to secondarily induced pathological changes in other organs (liver, muscles, hypophysis).

6. The observed facts indicate that the adrenal cortex (unlike the pancreas or hypophysis) is not preëminently involved in the maintenance of normal carbohydrate metabolism.

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BLOOD PRESSURE STUDIES ON INFANTS

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The lack of a standard method to measure the blood pressure of the new-born has produced widely varying results from different workers (1, 2, 3 and 4) and has prevented routine pressure measurements in infants. Systolic values between 75 and 90 mm. Hg were obtained by Neu in 1902 and Trumpp in 1906, who used the Gaertner tonometer (2, 5). The systolic pressures most widely cited at present (5, 6) range from 43 to 60 mm. Hg and were taken with cuffs 4 to 6 cm. wide by the Riva Rocci method or with an oscillometer.

These results must be questioned in view of the fact that average mean blood pressures (64 and 72 mm. Hg) obtained (5, 7) by cannulating the umbilical artery of the new-born are higher than the above systolic readings.

The study reported here, which has been reported in a preliminary form (8), was undertaken in order (*a*) to measure accurately the blood pressure at birth; (*b*) to establish a reliable method for clinical use in measuring the blood pressure of the new-born; (*c*) to study the effect of certain drugs and, (*d*) to record the pressure changes which occur under various physiological and pathological conditions.

METHOD. *Direct determination of umbilical pressures.* Arterial pressures were obtained by means of the "hypodermic manometer" of Hamilton, Brewer and Brotman (9). The needle of the manometer was inserted directly into an umbilical artery and the pressure pulses recorded photographically. From these records, the actual systolic and diastolic blood pressure could be measured within ± 1 mm. Hg.

When necessary spontaneous occlusion of the artery could be delayed by immersing the body and cord of the new-born in warm water.

Evidence that umbilical and brachial pressures are the same. It has been shown (10) that the systolic blood pressure in adults differs in different arteries. The tall narrow peaks of the pressure pulse curves from the femoral and particularly the dorsalis pedis arteries give systolic values much greater than the brachial systolic pressure. The pulse contours and

pressure values from the brachial artery differ very little from those of the axillary artery.

Which of these arteries does the umbilical resemble most closely in its pressure pulse contour? If the umbilical artery is tapped 37 cm. from the baby the curve is of the peripheral (dorsalis pedis) type. As shown in figure 1, (lower curve) the peaks are smooth, tall and narrow, and record systolic pressures which exceed by 11 mm. Hg simultaneous values from near the umbilicus. Pressure pulses from the central end of the umbilical artery (figs. 1, 6, 7 and 8) have contours very similar to those of the brachial artery (fig. 2). The peaks are wide and the anacrotic halt is present on the ascending limb of the curve. We have not been able to take simultaneous brachial and umbilical records; but we have been able to show that a modification of the Riva Rocci method, which we shall describe below, gave the same agreement with brachial records (fig. 2) from a ten day old child that it did with the umbilical records of the new-born. We, therefore, feel that umbilical records taken by puncture of a strongly pulsating cord near the umbilicus are a fair sample of the pressures in the central arteries of the baby.

Further confirmation is shown in figure 3, where a five month non-viable fetus showed the same systolic pressures in the left ventricle and in the umbilical artery.

Development of a clinical method. Immediately after taking the umbilical record, the brachial systolic pressure was measured by cuff and palpation at the wrist (auscultation was impractical). The use of the pediatrician's conventional cuff (4.5 cm. wide) gave results 20 to 25 mm. Hg too low. Trying narrower cuffs we found that we could get agreement with direct umbilical systolic values only if the cuff was 2.5 cm. wide (fig. 4). This is undoubtedly the clue to the low values of recent authors (1, 2, 3, 4) and to the fact that their readings are not in reasonable agreement with mean pressure determinations made by connecting the mercury manometer directly with the umbilical artery.

Observations were made upon 37 babies. Of these 24 were apparently normal, 8 had mothers with the toxemia of pregnancy and 5 were prematurely born.

Results. *Pressure studies on full term babies.* The umbilical pressure values of the 24 normal babies from normal mothers averaged 80.1/46.3 mm. Hg. The standard deviations of single observations were 8.1 (systolic) and 8.2 (diastolic). These pressures are markedly higher than those of the rabbit fetus (11).

The blood pressures of these full term new-borns compared with those of their mothers showed a correlation coefficient of only $+0.123 \pm 0.21$ for the systolic values and $+0.303 \pm 0.063$ for the diastolic values. These

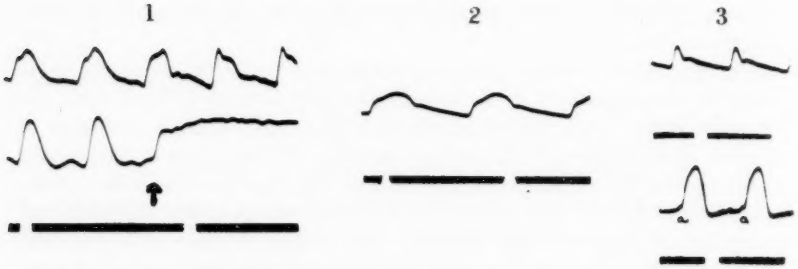

Fig. 1. Umbilical pressure pulse contours. Upper record: from the umbilical artery 1 cm. from the umbilicus, blood pressure (B.P.) = 88/51 mm.Hg. Clamping the cord just beyond the needle (↑) increased the pressure to 93/49 mm. Hg. Lower record: taken simultaneously from near the point of ligation (40 cm. from the umbilicus), B.P. = 99/50 mm. Hg. Time: 1 second intervals. The pulse velocity was 9 meters/sec. (linear).

Fig. 2. Brachial pressure pulses from a ten day old baby, B.P. = 94/65 mm. Hg, systolic pressure = 96 mm. with cuff 2.5 cm. wide and 73 mm. with cuff 4.5 cm. wide. Time: 0.5 second intervals.

Fig. 3. Umbilical and ventricular pressure pulses of a (non-viable) five month fetus. Upper record: umbilical, B.P. = 39/21 mm. Hg. Lower record: ventricular, B.P. = 39/1 mm. Hg. Auricular systole (*a*) increased the intraventricular pressure from 5 to 8 mm. Hg. Time: 1 second intervals.

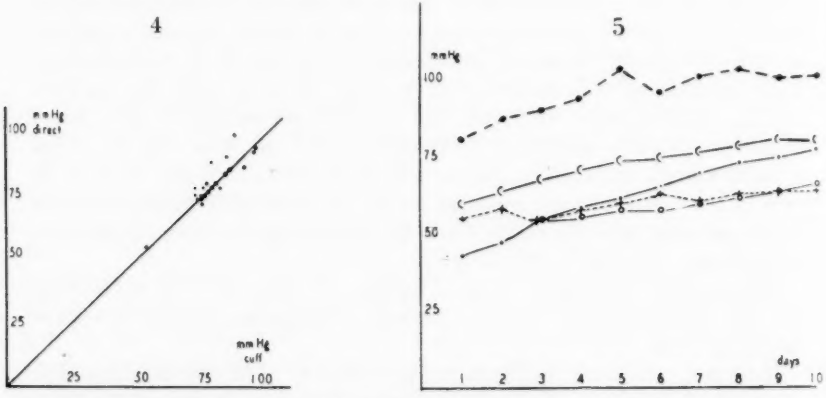

Fig. 4. A scatter diagram. Ordinates: systolic pressures of new-borns measured from the umbilical artery by a direct method. Abscissae: systolic pressures obtained from the brachial artery by the Riva Rocci method with a cuff 2.5 cm. wide. The diagonal line along which the points are scattered would represent identical results by both methods.

Fig. 5. Systolic pressures during the first 10 days of life. Ordinates: systolic pressures in mm. Hg. Abscissae: time in days. ·——· = our values; ⊂—— ⊂ = Ballard's values (1); +– – –+ = Rucker and Connell (4); ○ —— ○ = Seitz and Becker (2); and · —— · = Reis and Chaloupka (3).

figures indicate a slight correlation of the diastolic pressures and no significant correlation for the systolic pressures.

Pharmacological observations. The administration of obstetrical anesthesia to the mothers had very little, if any, effect upon the blood pressure of the babies. This corresponds to the effect in the mothers (12). The average pressures ranged between 78 and 83 mm. Hg (systolic) and between 41 and 48 mm. Hg (diastolic) whether the mother was unanesthetized, had ether, chloroform, morphine or scopolamine and a barbiturate.

Compared with adults, the drugs studied produced small pressure changes in the new-born. An intravenous injection of 0.2 mgm. of epinephrine HCl increased the umbilical pressure of one baby from 75/40 to 85/47 mm. Hg and the heart rate from 180 to 210 per minute. In two babies, inhalations of amyl nitrite reduced the umbilical blood pressure from 75/48 to 68/46 and from 80/51 to 70/40 mm. Hg. Their respective heart rates increased from 145 to 150 and from 160 to 170 per minute. The pulse contours did not show the characteristic changes seen in adults (13). Inhalations of a 30 per cent CO_2 and 70 per cent oxygen mixture by three infants increased respiration but did not cause any significant change in their blood pressure.

Physiological observations. Immediately after birth the cords of three babies were clamped at various distances beyond the needle of the manometer. Occlusion of the artery close to the umbilicus modified the pulse contour and increased the systolic pressure (fig. 1). The anacrotic halt on the ascending limb became a peak and usually registered the highest pressure during the systole. Occlusion of the artery at some distance from the needle of the manometer did not change either the contour or the blood pressure.

These phenomena have probably nothing to do with the stoppage of the placental circulation. Uterine contractions acting upon the placenta alone after the baby has been born have probably already reduced the placental circulation to a minimum.

The systemic arterial pressure levels do not change with the onset of respiration. Breathing, however, produces small rhythmic blood pressure undulations, which are caused by changes in the intrathoracic pressure.

Large changes in the intrathoracic pressure which occur during a cry (9) produce corresponding increases in the blood pressure. As seen in figure 6 the systolic and diastolic pressures were increased equally and returned to normal when the infant stopped crying. In ten babies the pressure increases ranged from 12 to 45 mm. Hg and averaged 27 mm. Hg.

Pressure over the region of the carotid sinus of three babies produced no significant change in the umbilical pressure pulses. This confirms Clark's finding in the cat and dog (14) that the carotid sinus reflexes are undeveloped at birth.

Pulsus alternans (ventricular alternation), a condition which so far as we know has never been described in infants, showed up clearly in two apparently normal new-born babies. As seen in figure 7 alternating beats differed in both systolic and diastolic pressures by 5 mm. Hg. The contours of the alternating beats showed the difference seen in the figure. Beats following a short diastolic interval and beginning at a high diastolic pressure showed a high systolic pressure in contrast to those following a long diastolic interval and low diastolic pressure. This indicates that the

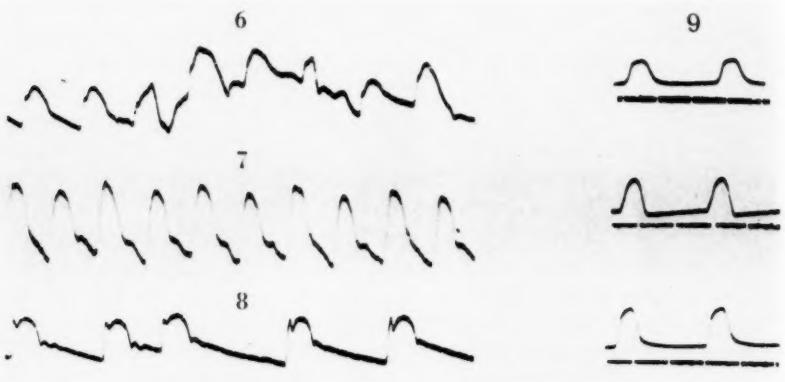

Fig. 6. Effect of crying on the blood pressure. Before the cry the arterial pressure was 65/35 mm. Hg. During the cry the systolic pressure reached 95 mm. Hg.

Fig. 7. Pulsus alternans in an apparently normal new-born. Alternating pulses differ in both systolic and diastolic pressure by 5 mm. Hg and in duration by 0.01 second.

Fig. 8. A premature beat in an apparently normal new-born. Systolic and diastolic pressures of each pulse were as follows: 90/50, 90/59, 97/44, 95/49 and 99/— mm. Hg. The heart rate (H.R.) was 100 per minute.

Fig. 9. Effect of epinephrine on the intraventricular pressure of a 5 month fetus with signs of terminal pulmonary edema. From above downwards: before drug injections, B.P. = 33/24 mm. Hg, H.R. = 24 per minute; one minute after an intravenous injection of 0.2 mgm. of epinephrine HCl, B.P. = 42/17, H.R. = 23; and one minute after an intravenous injection of 0.5 mgm. of epinephrine HCl, B.P. = 55/23, H.R. = 24.

infant's ventricle is quickly filled in contrast to certain anatomical deductions (6). The earlier beats are just as full as those that are delayed in spite of the rapid rate (156 beats per minute).

The same thing is shown in figure 8 where an extra systole occurs very early in diastole. This beat is nearly as strong as the regular beats preceding it and indicates again the fact that in the infant the venous pressure is such as to fill the ventricle very quickly.

Effect of age. During the first ten post-natal days the brachial systolic pressure gradually increases. This confirms the work of others (6). However, the pressure values are 20 to 40 mm. Hg higher than those previously reported (see fig. 5).

Effects of maternal toxemia. Umbilical records of eight babies of toxemic mothers showed the following pressures: 75/42, 78/48, 85/40, 85/45, 94/45, 100/60, 102/52 and 105/65. Comparing the mean of this group with the mean of the group born of normal mothers, we find the values to be respectively (systolic) 90.5 ±4. and 80.1 ±1.7 mm. Hg, (diastolic) 49.6 ±3.2 and 46.3 ±1.7 mm. Hg. The babies born of toxemic mothers probably have significantly higher systolic pressures (15).

In the "toxemic" series the average brachial systolic pressure increased from 91 mm. at birth to 95 mm. Hg on the third day (a rise of 4 mm.). In the "normal" series this increase was from 80 mm. at birth to 92 mm. Hg on the third day (a rise of 12 mm.).

These findings could be interpreted as favoring the view that there is a pressor substance in the blood of eclamptic patients which affects the infant's blood pressure through the placental circulation. After birth it would be slowly destroyed or excreted. This would explain the higher pressures at birth and the smaller average post-natal rise.

Pressure studies on premature babies. Records were taken from five premature babies. The fetal age in months with the systolic and diastolic pressures in mm. Hg were as follows: 5, 33/–;[1] 5, 39/21; 6½, 55/25; 7, 70/35; and 8, 75/45. These results confirm the statement that premature infants have low pressures corresponding to the length of gestation (3 and 5). The systolic values, however, are for reasons already mentioned 15 to 25 mm. Hg higher than those previously reported (3, 16). Following birth the systolic pressures of premature infants increased.

Intraventricular records (see fig. 9) were taken from a non-viable fetus when it showed signs of terminal pulmonary edema and was breathing irregularly and ineffectively. At this time the intraventricular diastolic pressure was 24 mm. Hg.

As shown in figure 9 intravenous injections of 0.2 and 0.5 mgm. of epinephrine hydrochloride caused relatively small pressure and heart rate changes. These results show that the five month fetus does respond, however poorly, to drugs which stimulate the sympathetic nervous system. Intramuscular injections of alpha lobeline (1.5 mgm.) and pituitrin (0.2 international unit) did not apparently change the blood pressure.

In one premature infant severe dehydration became evident twelve days after birth. The weight had decreased from 2360 grams to 1820 grams, and the systolic blood pressure had increased from 78 to 126 mm. Hg. Four hours after the subcutaneous injection of 150 cc. of 0.9 per

[1] From this fetus intraventricular pressure pulses were recorded.

cent saline, the systolic pressure was 112 mm. Hg. Two days later saline was again injected because the baby was still dehydrated. The systolic pressure decreased from 106 mm. to 92 mm. Hg within twelve hours. After the infant recovered from the dehydration the systolic pressure varied from 80 to 92 mm. Hg over a period of five days.

These results differ from the low values reported in the literature (5, 17, 18). This is very likely due to the fact that in this baby there were no signs of circulatory collapse. The baby overcompensated for reduced blood volume by vasoconstriction and had a higher blood pressure than normal. Regulation became normal again when dehydration was remedied. This response to dehydration was observed in three other infants whose ages were less than six months.

We are glad to express our thanks to Dr. Richard Torpin, of the Department of Obstetrics and Gynecology, for thorough coöperation in making available material for this study, and to acknowledge the help of Dr. Philip Dow, of the Department of Physiology and Pharmacology, for help in the statistical computations.

SUMMARY AND CONCLUSIONS

Optical registrations of arterial pressure pulses were obtained by the "hypodermic manometer" from 37 new-born babies. The same systolic values could be obtained by cuff and palpation if the arm band was 2.5 cm. wide. The conventional cuff of pediatricians gave systolic readings 20 to 25 mm. Hg too low.

The blood pressures of twenty-four full term new-borns averaged 80/46 mm. Hg. The standard deviation of a single observation was 8.1 mm. (systolic) and 8.2 mm. Hg (diastolic).

The infant's blood pressure was found to be not significantly affected by the following: 1, obstetrical anesthesia; 2, the onset of respiration; 3, clamping the cord after birth, and 4, administration of CO_2—O_2 to the baby.

The infant's blood pressure was affected slightly but significantly by the following: 1, blood pressure level of the (normal) mother $r = 0.303 \pm 0.063$ (diastolic); 2, toxemia of pregnancy (+10 mm. Hg systolic, +3 mm. Hg diastolic); 3, amyl nitrite administered to baby (−8 mm. Hg systolic, −7 mm. Hg diastolic), and 4, epinephrine, intravenously, 0.2 mgm. (+10 mm. Hg systolic, +7 mm. Hg diastolic).

The infant's blood pressure was markedly affected by the following: 1, crying (+10 to 45 mm. Hg systolic and diastolic); 2, dehydration without collapse (+30 mm. Hg systolic); 3, administration of fluid to dehydrated babies (−30 mm. Hg systolic); 4, age of infant, and 5, degree of prematurity.

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ERYTHROCYTE NUMBER IN YOUNG PIGEONS AND ITS RELATION TO HEREDITY, GROWTH AND METABOLISM

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The erythrocyte count of animals during early postnatal stages of life is relatively little known. The literature to 1926 was reviewed by Scarborough (1). The available data suggest that in some species (horse, cattle) there is a decrease, in other species (cat, dog, mouse, pig) an increase, in red cells from early infancy to adult life; but in general erythrocyte counts on the days immediately after birth are meager or missing. Studies by Sure, Kirk, and Walker (2) and Kindred and Corey (3) supply fairly satisfactory data for the rat; and Kunde *et al.* (4) have published adequate data for the rabbit. In both these species, as in the pigeon to be described here, a low initial blood count at birth is followed by a decrease during the first few days, and this in turn is quickly followed by a marked increase during growth and adolescence.

Nylin (5) studied relations of seasonal changes in oxygen capacity of the blood to seasonal changes in growth-rate and standard metabolism in a few Scandinavian children. Two earlier studies on birds are notable here. In small numbers of homozygous Frizzle fowl, where defective plumage and excessive rate of heat loss is associated with an increased respiratory metabolism, Landauer (6) found the erythrocytes similar in average number (but a greater variability) to those of normal fowl. Kalabukhov and Rodinov (7) counted erythrocytes in a few birds (26 sparrows; 12 gulls) aged 1 to 30 days (also studied white mice and ground squirrels) and partially related the blood count (and Hb) to age and growth changes. An increase in the number and a decrease in size of erythrocytes with early age (data for days 1 to 5 lumped together) and with increase in body weight was reported for the several species studied. We are aware of no study of erythrocyte number in hybrid young and in their parent races, nor of additional previous attempts to associate early postnatal changes in the blood count with concurrent changes in growth rate and basal metabolic rate. We have attempted to obtain data from young pigeons concerning these points.

MATERIALS AND METHODS. Two quite distinct races—Tipplers and Homers—were selected as principal material, and the individuals used

had been inbred for at least 6 to 10 generations. Hybrids (first to sixth generations) from these two races completed the material needed for a very superficial view of the behavior of erythrocyte number in hybridization. An additional mixed group, here called *X* and composed of certain other races and hybrids, was used as a further and independent test of possible numerical change of erythrocytes during various periods of preadult life. Sex was not recorded but both males and females were included among the young chosen at random. Cell counts were made at 13 different dates after hatching (by our method of reckoning age the pigeon is already 18 days old at hatching), namely, on days 1, 2, 3, 4, 7, 10, 15, 20, 30, 40, 50,

TABLE 1

Changes in erythrocyte number (in thousands per cubic millimeter) in four races of pigeons from day of hatching (at 18–19 days) to a period 100 days later

The number of birds used in the determination of each erythrocyte value is indicated.

KIND OF PIGEON	ERYTHROCYTES (THOUSANDS) AT VARIOUS AGES (IN DAYS) INDICATED													TOTAL
	19	20	21	22	25	28	33	38	48	58	68	93	118	
Tippler	1358	1257	1168	1288	1497	1771	2193	2532	3186	3460	3471	3464	3222	
	23	12	23	17	20	21	18	19	20	27	28	24	17	269
Tippler ×	1274		1069	1218	1495	1735	2005	2375	3183	3463	3428	3287	3118	
Homer	9		12	2	13	12	10	11	10	8	10	5	4	106
Homer	1241	1178	1009	1109	1352	1658	1919	2313	2996	3436	3407	3259	3033	
	18	3	15	6	15	13	16	16	14	16	9	5	5	151
X	1228	1104	1067	1101	1412	1662	1956	2336	2857	3280	3314	3307	3104	
	10	18	24	17	35	33	27	28	29	22	22	21	10	296
Total.....	60	33	74	42	83	79	71	74	73	73	69	55	36	822

75 and 100. In the graph and table therefore a bird at 100 days after hatching is regarded as 118 days old.

In part, but only in part, counts were made on the same birds throughout this period, and the number of counts per period per racial group varied (with 2 exceptions) from 5 to 30. The very high degree of consistency evident in the several average values obtained—particularly in the two groups of genetically uniform material (see table 1)—indicates that individual variation has been satisfactorily smoothed by a sufficient number of cell counts. Duplicate counts in a Levy-Hauser counting chamber on blood from the squab's toe were made with the staining and other technique used by Riddle and Braucher (8) for counts on adult birds of these and other races of pigeons. Since the blood of adult pigeons was earlier

found to show marked seasonal changes (8) in the cell count the present study was confined to the warm period of late spring and summer.

RESULTS. 1. *Changes in erythrocyte count with age.* The data of table 1 show that from the first to the third day after hatching the cell count progressively decreased; for the 4 racial groups we obtain mean values of 1,275,200 on first day, 1,179,700 on second day, and 1,078,200 on third day. An increase is found on the fourth day (1,179,000) and continuously thereafter for about 35 days when the highest concentration (3,409,700 cells per cmm.) of the whole life cycle is attained. From the fortieth day to about the seventy-fifth day after hatching erythrocyte number is maintained at its highest level and thereafter (within a month) it rather rapidly decreases by approximately 10 per cent to values characteristic of adult life. At 100 days after hatching the cell count of Tippler pigeons is still nearly 8 per cent higher than that found by Riddle and Braucher for 20 adults of this race in summer.

The curve which describes the cell count in the early postnatal life of the pigeon thus has three (or four) distinct parts. The first two segments of this curve (fig. 1) are remarkably similar to results obtained by Kindred and Corey (3) in the albino rat whose cell count decreases during the first three days (this not observed by Sure *et al.*) of postnatal life and thereafter for a short period (apparently 2 weeks) rapidly increases. It seems that the cell count in the young or adolescent rat never exceeds that of the adult. In the rabbit also Kunde and associates (4) found high erythrocyte (and Hb) values at 1 day, lowest at 3 days and 2 weeks; thereafter a progressive increase to 18 weeks, highest values being maintained between the 18th and the 22nd weeks, with a subsequent decline in adult life. Numerous data for the human, as summarized by Goldhamer (9), indicated higher red cell count at birth and the first two days than at 5 to 12 days and later life; but the difference is not striking. Likewise the hemoglobin value seems highest during the first two days, "being lowest from the third month to the second year, there is then a slow return to the adult range with a slight dropping again at puberty."

Kindred and Corey, and others, suggested loss of blood at birth, from umbilical vessels and within the placenta, as the chief source of a decrease of cell number during the first three days. Since the diminution is progressive during a three-day period, and since a quite comparable change is now demonstrated in a bird, we consider it more probable that the diminution is associated with the initiation of pulmonary ventilation of the blood and with a temporarily decreased body temperature (and metabolism) incident to birth and hatching in these two animals. Storch (10) reported a few measurements which indicated that lambs of 2 weeks have a count 2,000,000 lower, and those of two months 2,500,000 higher, than that of adult sheep. Four counts made by Juhn and Domm (11)

on one-day chicks indicate notably higher values than were obtained from four counts made 18 days later. These citations thus suggest that a very temporary decrease of the blood count following birth or hatching may be the rule in both birds and mammals.

2. *Changes in erythrocyte count in relation to growth.* The growth curve of Tippler (and other) pigeons was obtained in an earlier study (12). That curve is reproduced in figure 1 alongside the curve which describes change in the red cell count with age in this same race of pigeons. It will

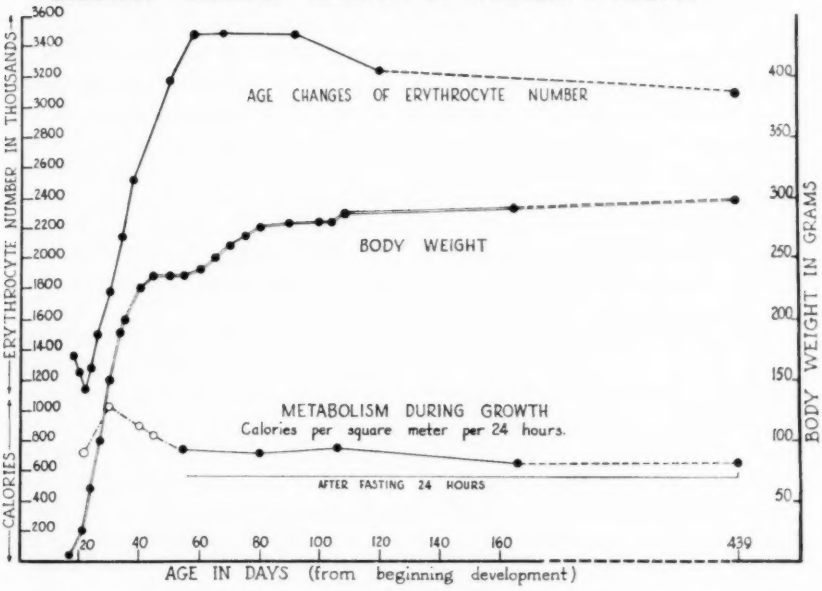

Fig. 1

be noted that in general the curves are parallel. Only during the very short postnatal period of decrease of red cells are the two curves essentially different. The data of others for the rat and rabbit would seem to coincide closely with those here found in the pigeon, though the available data for horses and cattle indicate quite the reverse relationship.

3. *Changes in erythrocyte count in relation to basal metabolism.* On figure 1 is also shown the curve earlier obtained (13) for the respiratory metabolism (at 30°C.) of Tippler pigeons at all stages of growth and life. The four open circles on this curve represent basal values as these were calculated (13) from values obtained on non-fasting young. It is evident that the metabolism markedly increases during the first half of the period

of extremely rapid growth, and during the initial one-fourth of the period of rapid increase of red cells; thereafter the metabolism decreases rapidly for a short period (20 or 25 days) though both body weight and erythrocyte number continue to increase. From about 38 days after hatching the B.M.R. of these pigeons very slowly decreases throughout the rest of life; a similar slow decrease in erythrocyte number begins about two months later.

In adult pigeons an earlier study (8) has shown that the number of erythrocytes increases from summer to autumn, and similarly the B.M.R. (measured at 20°–30°) was found (14) to be higher in autumn than in summer. In children about 6 years old, Nylin (5) reported that the season of maximum oxygen capacity of the blood does not coincide with the season of maximum B.M.R. but does coincide with the season of greatest height-increase. From the considerable literature dealing with the relation of hyperthyroidism to the red cell count we note that in a series of 600 cases of hyperthyroidism Jackson (15) found no essential variation from the normal. In a large group of adult male schizophrenic patients in which erythrocytes were slightly below normal Hoskins and Jellinek (16) found this condition associated with a B.M.R. 16 per cent below prediction; in these cases dosage with desiccated thyroid resulted in a significant increase of red cells.

4. *Influence of heredity on erythrocyte number.* The Tippler pigeons show (table 1) a mean erythrocyte count 7.0 per cent higher than the Homers, and hybrids from these two races gave a mean erythrocyte count 3.6 per cent lower than that of the Tipplers and 3.4 per cent higher than that of the Homers. That the observed difference between the hybrids and their parent races is statistically significant is satisfactorily indicated by the fact that at only one of the 13 ages measured was the value found for the hybrids not intermediate to those of the two parental races; and in this partial exception (it remained higher than the parent race having the lower value) the value obtained in the hybrids exceeded by only 3,000 cells that of the parent race (Tipplers) having the highest cell count. Since the hybrids studied were from generations F_1 to F_6, and erythrocyte number was known in none of their own (hybrid) parents, these data merely show that the erythrocyte number of this population of hybrids was influenced by the racially characteristic erythrocyte levels of both of the parental races. The very great change of the cell count with age (1,078,000 to 3,409,700) is equally apparent in hybrids and in their parental races.

SUMMARY

On four racial groups of pigeons studied 1 to 100 days after hatching 822 erythrocyte counts were made during summer months. Changes in

erythrocyte number in their relation to previously observed changes in growth rate and metabolic rate are shown by graph.

During the first three days of postnatal life the number of red cells decreased from 1,275,200 to 1,078,000 per cubic millimeter. About 27 days later red cells attained a maximum (3,409,700) which was maintained for about 30 to 35 days. During the following month, and coincident with the termination of notable body growth, the cell count was rapidly diminished by about 10 per cent.

Reduction of the number of red cells during the very first days of postnatal life has now been observed in four species: rat, rabbit, man and pigeon. A further study of the significance of this change and of its possible occurrence in other species is needed.

Erythrocyte number in young pigeons is rather closely associated with growth capacity except during the first three days of postnatal life.

Only the first one-third of the period of erythrocyte increase is associated with a continuing increase of basal metabolic rate. After the end of the third or fourth month both red cells and B.M.R. slowly diminish throughout life.

The number of erythrocytes found in hybrids from Tippler and Homer pigeons was found to be significantly lower (3.6 per cent) than that of Tipplers and greater (3.4 per cent) than that of Homers. This influence of heredity on erythrocyte number in pigeons was evident at hatching and during the several later stages of life.

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THE EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON THE VITAMIN B_1 CONTENT OF SOME RAT TISSUES

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Himwich, Goldfarb, and Cowgill (1) reported that in dogs fed desiccated thyroid gland an increased amount of undifferentiated vitamin B_1 was needed. Sure and Buchanan (2) claim that vitamin B_1 is an excellent antithyrogenic agent, as judged by the weight maintenance of rats receiving thyroxin, after the stable components of the B complex have been provided for. Later Drill (3) was also able to prevent hyperthyroidism and the subsequent loss of weight and of liver glycogen by feeding large amounts of yeast. No report of the vitamin B_1 content of the tissues of hyperthyroid animals has yet appeared in the literature. From the apparent need of vitamin B_1 during experimental hyperthyroidism one might expect a marked lowering of the vitamin B_1 content of the animal tissues during experimental hyperthyroidism. The vitamin B_1 content of some of the tissues of normal and hyperthyroid animals was therefore determined.

METHODS. Recently Schultz, Atkin, and Frey (4) have developed a method for the determination of vitamin B_1, which they have applied to tissue work. Their method depends on the stimulation of the fermentation of yeast cells, the acceleration being proportional to the amount of vitamin B_1 present, and the subsequent measuring of the volume of CO_2 produced. The only interfering substance the authors have found are pyrimidine compounds and products formed in the process of autoclaving yeast, when the vitamin B_1 is destroyed. Their method checks very well with the rat assay method and allows the determination of vitamin B_1 to the 0.1 gamma, with a reported accuracy of 0.03 gamma (4).

The rats were raised on Purina Dog Chow and for the experiment were transferred to diet no. 3, consisting of: cod liver oil, 2; salts, 4; Crisco, 10; casein, 20; cornstarch, 59; and yeast, 5. The yeast (Fleischmann's no. 15190) contained 18 international units of vitamin B_1 per gram and 20 Sherman-Borquin units of vitamin B_2 per gram. The control rats (group 1) received 12 grams of diet no. 3 per day with water ad lib, giving each rat about 10.8 I. U. of vitamin B_1 per day and about 12 S-B units of vitamin B_2 per day. The experimental animals (group 2) also received

12 grams of diet no. 3 per day plus 100 mgm. of desiccated thyroid gland per day. The group 2 rats soon lost weight when the thyroid feeding was started. The thyroid gland was fed for 17 days during which time the females had lost an average of 24 grams and the males an average of 47 grams, and some of the hyperthyroid animals had died. At this time, while some of the hyperthyroid animals were near death, the rats in groups 1 and 2 were chloroformed and the liver, kidney, spleen, and heart dissected for vitamin B_1 determinations. The whole spleen, kidney,

TABLE 1

Vitamin B_1 content of tissue in gammas per gram of tissue

GROUP 1 (CONTROL RATS)							GROUP 2 (HYPERTHYROID RATS)						
Rat number	Sex	Heart	Kidney	Spleen	Liver	Muscle	Rat number	Sex	Heart	Kidney	Spleen	Liver	Muscle
1	F	6.7	4.8	1.3	5.0		6	F	5.0	2.3	1.2	2.7	
2	F	5.9	4.4	1.0	5.4		7	F	5.6	2.1	1.1	1.9	
3	F	5.6	3.5	1.2	4.8		8	F	5.1	3.4	0.4	2.2	
4	M	5.4	2.7	1.3	4.1		9	M	1.5	1.6	0.8	2.0	
5	M	5.3	2.5	1.1	4.9		10	M	3.9	2.0		1.6	
							11	M	2.9	1.8	0.6	1.6	
Average.....		5.8	3.6	1.2	4.8		Average....		4.0	2.2	0.8	2.0	
GROUP 3 (CONTROL RATS)							GROUP 4 (HYPERTHYROID RATS)						
12	F	7.8	10.4	3.5	14.1	2.6	17	F	10.8	6.5	2.3	4.5	
13	F	8.9	9.2	4.7	11.7	2.3	18	F	9.2	7.0	2.6	4.5	
14	F	7.0	8.5	2.7	11.0	2.7	19	F	10.5	6.3	2.5	4.8	2.4
15	F	9.6	10.3	2.8	8.3	1.4	20	F	12.2	6.6	3.2	6.0	2.2
16	M	8.8	8.6	1.5	9.0	2.1	21	M	8.4	5.5	2.4	4.6	
							22	M	10.5	6.5	2.9	6.4	2.6
							23	M	12.3	6.7	2.9	5.5	2.2
Average.....		8.4	9.4	3.0	10.8	2.2	Average....		10.6	6.4	2.7	5.2	2.4

or heart was weighed, ground with sand, and washed into Erlenmeyer flasks. The contents were made slightly acid to litmus and brought to a boil for a minute. They were stored in a refrigerator until vitamin B_1 was determined. The whole liver was weighed, ground (without sand) and a sample weighed for vitamin B_1 determination. The results are given in table 1.

Leong (5), in a study of the vitamin B_1 content of normal rat tissues, has shown that the vitamin B_1 storage increases and approaches a max-

imum with an increased *oral* intake of vitamin B_1. The experiment was therefore repeated with rats receiving a higher intake of vitamin B_1. Any lowering of the vitamin B_1 content of the tissues of hyperthyroid rats should be more pronounced at a higher storage level.

The rats in group 3 were normal rats receiving 12 grams of diet no. 3 per day. The rats in group 4 also received 12 grams of diet no. 3 per day plus 100 mgm. of desiccated thyroid gland per day. After the thyroid gland has been fed for 8 days the females in group 4 had lost an average of 18 grams and the males an average of 32 grams while the control group gained in weight. On the 9th day each rat in groups 3 and 4 received in addition to the above diet, 500 gamma of crystalline vitamin B_1 injected subcutaneously per day. The thyroid gland was still being fed in group 4. After injecting the normal and hyperthyroid animals with 500 gamma of vitamin B_1 for 9 days (with a total of 17 days' thyroid feeding in group 4) the rats were chloroformed and the tissues dissected for vitamin B_1 determination. The results are given in table 1.

Quantitative 24 hour samples of urine were collected at various intervals from the rats in groups 3 and 4 while they were being injected with the 500 gamma of vitamin B_1, to see if there was a diminished output of vitamin B_1 during hyperthyroidism.

Discussion. The hyperthyroid rats in group 2 show normal amounts of vitamin B_1 in the spleen and a reduction of vitamin B_1 content in the liver and kidney. The females in group 2 show normal amounts of vitamin B_1 in the heart, whereas in the males the vitamin B_1 content of the heart is reduced. This difference in the vitamin B_1 content of the heart of the males and females of group 2 is unexplained. Although, it has been noticed in this laboratory that the female rat is more resistant than the male to thyroid feeding, as judged by the loss of weight, and the greater loss of weight of the above males might account for the lower values of vitamin B_1 in the heart.

In groups 3 and 4, where the animals were injected with 500 gamma of vitamin B_1 for 9 days, the hyperthyroid rats show normal values for vitamin B_1 in the spleen and muscle, with a reduction in the kidney, and a marked reduction in the liver. The hearts of the hyperthyroid animals are slightly above the controls. Group 4 is distinguished from group 2 by the large amount of vitamin B_1 available, and since the heart during hyperthyroidism is much more active than normal, more vitamin B_1 might be needed and be used by the more active heart muscle.

Leong (5) has shown that the muscle and liver of the rat contain 80 to 90 per cent of the body stores of vitamin B_1. The hyperthyroid rats of group 4 show a normal amount of vitamin B_1 content of the muscle and approximately a 50 per cent loss of vitamin B_1 per gram of liver when compared with group 3. When the vitamin B_1 in the liver is calculated

per weight of whole liver per 200 grams weight of rat, an average total of 61.8 gamma of vitamin B_1 for group 4 is obtained as contrasted with an average value of 96.1 gamma of vitamin B_1 for group 3, showing a reduction of 35.7 per cent storage in the liver. Leong (6) also found that a certain amount of the vitamin B_1 of the normal rats, depending on the intake of vitamin B_1, was probably destroyed during metabolism. The normal and hyperthyroid animals tested eliminated the same amount of vitamin B_1 (average of 425 gamma/day) in the urine while being injected with 500 gamma of vitamin B_1 per day. Increased metabolism produced by thyroid feeding does not increase the amount of vitamin B_1 destroyed by body metabolism, as measured by the vitamin B_1 eliminated by the urine. Does this mean that the amount of vitamin B_1 needed by the rat is not increased during hyperthyroidism, that the only effect of hyperthyroidism, with respect to vitamin B_1, is to reduce the storage level of vitamin B_1 in some of the rat tissues? This question cannot be answered until it is known whether the vitamin B_1 eliminated in the urine has done work in the body. The possibility also remains that vitamin B_1 which has done work in the body is eliminated in a slightly different form, that is not differentiated by this test. From the apparent need of the rat for vitamin B_1 during hyperthyroidism it might appear that the vitamin B_1 eliminated has done work in the body, but this question must remain open.

The hyperthyroid rats of group 4 still continued to lose weight, and some died, when injected with the 500 gamma of vitamin B_1 per day. Even though the tissue storage of vitamin B_1 of group 4 is below that of group 3, the control rats receiving 500 gamma of vitamin B_1, it is still above that for the normal rats in group 1, receiving yeast with no added vitamin. Thus a lack of vitamin B_1 per se, is not responsible for the death or continued loss of weight of the hyperthyroid rats. It should be noted that the above experiments on hyperthyroidism were performed with a constant calorie intake, namely, 12 grams of diet no. 3 per day. Any curative action of vitamin B_1 on experimental hyperthyroidism will probably depend on calorie intake (1). This is being investigated.

SUMMARY

1. Rats receiving 12 grams of a normal diet per day plus 100 mgm. of thyroid gland per day showed a normal amount of vitamin B_1 in the spleen, a reduction in the kidney, and a marked reduction in the liver, when compared with normal rats on the same diet.

2. Hyperthyroid rats receiving 12 grams of normal diet per day and later injected with 500 gamma of vitamin B_1 per day, while still receiving the thyroid gland, showed normal amounts of vitamin B_1 in the spleen and muscle, slightly raised content in the heart, and a definite reduction in the kidney and liver, when compared with normal rats receiving 500

gamma of vitamin B_1 per day. The vitamin B_1 content of the whole liver per 200 grams weight of rat was reduced 35.7 per cent in the hyperthyroid rats.

3. The hyperthyroid rats injected with 500 gamma of vitamin B_1 per day eliminated the same amount of vitamin B_1 in the urine as normal rats receiving 500 gamma of vitamin B_1.

4. The deaths of some of the rats and continued loss in weight of the hyperthyroid rats in group 4 is not due to a lack of vitamin B_1 per se, for the vitamin B_1 content of the tissues of group 4 is still above that of the normal animals in group 1.

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CHANGES WITH AGE IN THE BLOOD PRESSURES IN ADULT MEN[1]

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Observations of the level of the blood pressure in normal persons have led to the accumulation of more measurements of this function than of that of any other in the human body. In the early and middle periods of life what the usual limits are and the average level during ordinary daily activity, have been much investigated. But after the age of sixty years, adequate studies have been rare; the common practice of pooling observations made beyond this age has unfortunately obscured the course which the events follow. There is, therefore, still need for detailed analysis of the behavior of the blood pressures in later life, and for learning more exactly the range of their variability. This study makes an attempt to supply this need.

The same hundred men, twenty in each decade from forty to eighty-nine years, other of whose functions have also been studied, were the subjects of this analysis. There were also two men, ninety-one years, and one, one hundred and one years of age. These men were all healthy.

METHODS. The conditions under which the examinations were made have already been described (1). The measurements were made with a mercury manometer by the auscultatory method (2). The air-cuff was fastened about the upper arm and the bell of the stethoscope was placed over the brachial artery at the antecubital space.

The subjects were in a basal state—at rest in bed, in the morning, having fasted 14 hours. Readings were obtained from both arms. Differences between the two were noticed in some subjects. The systolic and diastolic pressures were in most patients higher in the right arm. The average increase in systolic pressure at five-year intervals of age, varied from 4 to 9 mm. Hg; and in diastolic level, 0 to 8 mm. (Lewis (3), table 1).

The figures obtained from measuring the pressures in the right arm were selected for analysis. Those from the left showed changes at different ages similar to those of the right.

RESULTS. The results (Lewis (3), table 1) are presented under the headings, the systolic pressure, the diastolic pressure, the mean pressure

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TABLE 1

Summary of results of the statistical analyses of the blood pressures based on data in table 1 (Lewis (3))

AGE GROUP	SYSTOLIC BLOOD PRESSURE					DIASTOLIC BLOOD PRESSURE					MEAN BLOOD PRESSURE					PULSE PRESSURE				
	Mean M_{SP}	Standard deviation σ_{SP}	Standard error of mean $\sigma_{M_{SP}}$	Coefficient of variation $\frac{100\sigma_{SP}}{M_{SP}}$	Coefficient of correlation $r_{A\cdot SP} \pm \sigma_r$	Mean M_{DP}	Standard deviation σ_{DP}	Standard error of mean $\sigma_{M_{DP}}$	Coefficient of variation $\frac{100\sigma_{DP}}{M_{DP}}$	Coefficient of correlation $r_{A\cdot DP} \pm \sigma_r$	Mean M_{MP}	Standard deviation σ_{MP}	Standard error of mean $\sigma_{M_{MP}}$	Coefficient of variation $\frac{100\sigma_{MP}}{M_{MP}}$	Coefficient of correlation $r_{A\cdot MP} \pm \sigma_r$	Mean M_{PP}	Standard deviation σ_{PP}	Standard error of mean $\sigma_{M_{PP}}$	Coefficient of variation $\frac{100\sigma_{PP}}{M_{PP}}$	Coefficient of correlation $r_{A\cdot PP} \pm \sigma_r$
years	*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*		*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*		*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*		*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*	
40–44	116	10.7	3.6	9.3	−0.2529															
45–49	121	8.4	2.8	6.0	−0.4006	76	8.8	2.9	11.5	−0.0465	96	9.3	3.1	9.7	−0.1641	39.5	5.8	1.9	14.7	−0.3931
50–54	123	16.0	5.3	13.1	−0.1926	78	8.8	2.3	8.7	−0.1423	99	7.0	2.3	7.0	−0.3121	42.4	6.4	2.1	15.2	−0.3711
55–59	126	21.0	7.0	16.7	+0.2386	77	10.0	3.3	13.1	+0.1575	100	12.2	4.1	12.2	−0.0520	45.9	11.2	3.7	24.4	−0.4171
60–64	124	18.8	5.4	15.0	−0.2119	83	12.8	4.3	15.5	+0.1770	109	17.7	5.9	16.2	−0.1557	43.0	12.6	4.2	29.4	+0.2173
65–69	134	11.7	4.8	8.7	−0.3383	75	9.0	2.6	12.0	−0.2156	100	13.3	3.8	13.3	−0.2821	48.8	12.4	3.5	25.4	−0.1644
70–74	141	22.7	6.5	16.0	+0.3277	75	10.2	4.2	13.5	−0.4345	105	10.1	4.1	9.6	−0.1733	58.9	9.5	3.9	16.2	+0.0503
75–79	153	18.3	7.5	11.9	+0.0520	76	10.4	3.0	13.7	+0.3206	109	16.2	4.7	14.9	+0.5128	65.4	15.3	4.4	23.3	+0.2681
80–84	154	21.3	5.9	13.8	+0.0750	85	11.0	4.5	12.9	+0.2351	119	13.0	5.3	10.9	−0.0324	68.0	14.8	6.0	21.8	−0.1103
85–89	158	26.6	11.9	16.8	+0.5414	76	10.9	3.0	14.3	+0.6263	115	15.1	4.2	13.1	+0.1977	77.9	14.9	4.1	19.1	−0.4310
90–94	147	23.0		15.6		78	6.4	2.8	8.2	+0.3885	118	15.6	7.0	13.3	+0.5102	82.0	21.5	9.6	26.2	
100–104	136					75	5.0		6.6		111	14.0		12.6		72.0	18.0		25.0	
40–49	118	10.2	2.3	8.6	+0.1056	77	7.9	1.8	10.3	+0.0462	98	8.4	1.9	8.6	+0.0349	41.0	6.3	1.4	15.4	−0.0196
50–59	124	18.9	4.3	15.2	+0.1590	80	11.9	2.7	14.9	+0.3000	105	15.9	3.7	15.2	+0.1991	44.5	12.0	2.8	27.1	−0.1245
60–69	128	17.3	4.0	13.6	+0.0980	75	9.3	2.1	12.4	−0.2470	101	12.4	2.9	12.3	−0.0241	52.3	12.5	2.9	23.9	+0.3195
70–79	146	22.0	5.0	15.1	+0.3380	79	11.5	2.6	14.5	+0.4445	112	15.9	3.7	14.2	+0.3984	66.3	15.2	3.4	22.9	+0.1557
80–89	155	23.2	5.3	15.0	+0.2731	77	9.6	2.2	12.6	+0.2628	116	15.3	3.5	13.2	+0.2386	79.1	17.3	4.0	21.9	+0.1498
90–101	143	19.6	13.9	13.7	−0.2305	71	6.8	4.8	9.5	−0.6667	107	12.6	8.9	11.7	−0.4074	72.0	14.7	3.4	20.4	+0.4284
40–64	122	16.3	2.2	16.3	+0.1577	78	10.0	1.4	12.9	+0.0043	101	13.4	1.8	13.3	+0.1476	44.2	10.8	1.5	24.4	+0.2335
65–89	148	22.5	3.3	15.2	+0.3847	78	10.6	1.6	13.7	+0.0777	113	15.4	2.2	13.6	+0.3091	70.6	17.3	2.5	24.5	+0.4637
65–101	148	22.3	3.2	15.1	+0.2705	77	10.5	1.5	13.6	−0.0347	112	15.2	2.2	13.6	+0.1883	70.7	17.2	2.4	24.3	+0.3846
40–89	134.2	23.4	2.3	17.4	+0.5909 ±0.0657	77.7	10.3	1.3	13.2	+0.0097 ±0.1010	106.4	15.4	1.5	14.5	+0.4279 ±0.0825	56.6	19.4	1.9	34.3	+0.7142 ±0.0495
40–101	134.4	23.3	2.3	17.4	+0.5785 ±0.0662	77.5	10.2	1.0	13.2	−0.0346 ±0.0994	106.4	15.4	1.5	14.4	+0.3995 ±0.0836	57.1	19.5	1.9	34.1	+0.7022 ±0.0504

and the pulse pressure. Each of them is described under several aspects.

1. *The systolic pressure.* a. The systolic pressure rose continually after age 40 but the greatest rise was observed after age 65 (table 1, fig. 1). At age 40 to 44, the average pressure was 116 mm.; at age 60 to 64, it was 124 mm., an increase of only 8 mm. in the twenty-five year span. At age 85 to 89, it was 158 mm. a further increase in this twenty-five year span of 34 mm. The increase approximately quadrupled. For the men over 90 years the pressure was actually lower.

The scatter of individual measurements was greater in the older groups than in the younger (figs. 2, 3). The lowest systolic pressure observed was

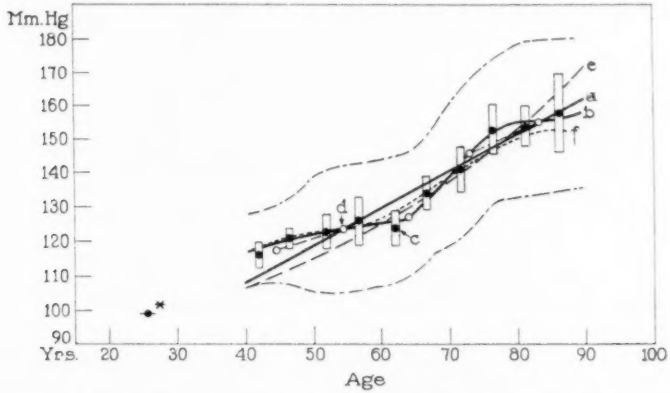


Fig. 1. Systolic blood pressure in normal men over 40 years of age under basal conditions.

a, straight line regression $Y = a + bX$. *b*, smoothed curve of 5 year averages with limits---------- of standard error of estimate for each decade. *c*, curve of 5 year averages, ± standard error of mean of age, and of systolic blood pressure. *d*, curve of 10 year averages. *e*, calculated curve of regression $Y = \frac{1}{a + bX}$. *f*, smoothed curve of 10 year averages.

90 mm., in the right arm of a man of 57 years (Y. M. C. A. secretary); the reading from the left arm was 100 mm. The highest was 195 mm. in a man of 85 years in whom there was detected no evidence of obvious abnormal anatomical change. The inclusion of subjects whose systolic level is about 160 mm. heretofore considered the upper normal limit, will be given further consideration in the discussion.

The deviation from the mean in the various age classes was somewhat greater in the elderly (table 1, figs. 2, 3). The largest standard deviation was 26.5 mm. in the men aged 85 to 89 years, the smallest 8.4 mm. in

those aged 45 to 49. The percentage deviation of the mean (coefficient of variation) varied from 6.0 to 16.8 per cent and was equally large in the 55 to 59 year, 70 to 74 year, 85 to 89 year classes. For the entire series, the standard deviation was 23.3 mm. (17.4 per cent of the mean of 134 mm.).

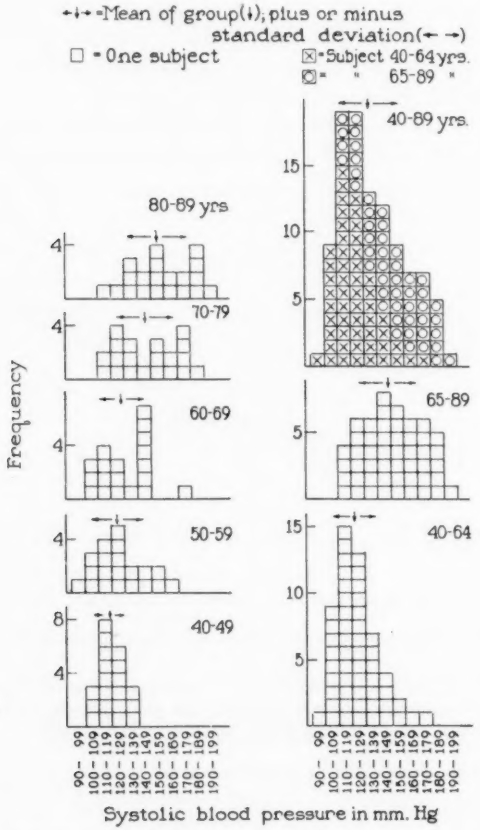


Fig. 2. Distribution of the subjects at various age groups indicating the frequency, the mean ± one standard deviation, and the mode of the systolic blood pressure.

b. The level of the median of the systolic pressure increased gradually with age. This fact emerges from a study of figure 2. So did the mode, at earlier ages; but in the decades 70 to 79 and 80 to 89 years, with the appearance of more individuals with higher pressures, the shift of the mode to high levels ceased, because of the greater scattering of the measurements.

If all subjects are divided into two groups the mode of the subjects, aged 40 to 64 years, was 110 to 119 mm. Hg; of those aged 65 to 89 years, 140 to 149 mm. Hg.

c. As has already been said the level of the means of the systolic pressure of the five or ten year age classes rises with increasing age (table 1, fig. 1).

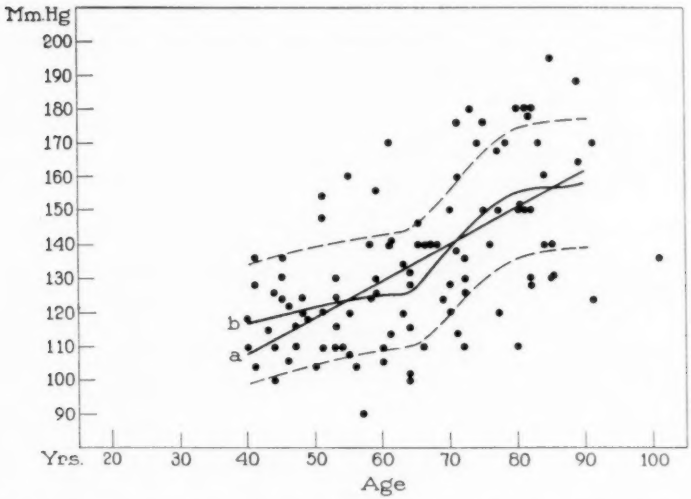


Fig. 3. Systolic blood pressure in normal men over 40 years of age under basal conditions.

●, individual pressure. *a*, straight line regression, $Y = a + bX$. *b*, curvilinear regression, smoothed curve of means of 5 year classes, with limits----------of standard error of estimate for men aged 40 to 89 years.

The linear equation which expresses this relationship, from 40 to 89 years, is

$$\overline{SP} = 65.057 + 1.083 \times A$$

where $\overline{SP}$ = systolic pressure in mm. Hg and
A = age in years

When $A = 40$, $\overline{SP} = 108$;
$A = 90$, $\overline{SP} = 162$ (figs. 1, 3)

The actual increase during this period of fifty years is accordingly 54 mm. Hg, or 50 per cent; for each decade it is therefore 5.4 mm. or 5 per cent.

The coefficient of correlation, $+0.5909 \pm 0.0657$, indicates that the linear relation (tables 1, 2) of the two is highly significant. If the results of the entire series, including the three men over 89 years of age, are cor-

related with age, the coefficient $+0.5875 \pm 0.0662$ is likewise significant (table 1). The equation then is:

$$\overline{SP} = 74.54 + 0.926 \times A$$

When $A = 40$, $\overline{SP} = 112$;
$A = 90$, $\overline{SP} = 158$;
$A = 100$, $\overline{SP} = 167$.

The actual increase and rate of increase are slightly less than in the series of 100 men, distributed evenly by decades.

d. The general trend of the systolic pressure is a rise and is directly related to age. But the mean values, if taken by five or ten year periods, present not a straight line relation but a curvilinear one. In this curve (figs. 1, 3) there is first a gradual rise from 40 to 64 years and then a rapid

TABLE 2

Correlations relating blood pressures and age in 100 men aged 40 to 89 years

BLOOD PRESSURE	GRAPH	COEFFICIENT OR INDEX OF CORRELATION ± STANDARD ERROR	VALUE OF t	COEFFICIENT OR INDEX OF DETERMINATION	STANDARD DEVIATION OF CALCULATED VALUES	STANDARD ERROR OF ESTIMATE
				per cent	*mm.* Hg	*mm.* Hg
Systolic	a	**+0.5909** ±0.0657	9.0	34.9	13.8	19.1
	b	**+0.6317** ±0.0613	10.3	39.9	14.8	18.7
	c	**+0.5868** ±0.0662	8.9	34.4	13.7	18.9
Diastolic	a	**+0.0097** ±0.1010	0.096	0.007	0.1	10.3
Mean	a	**+0.4279** ±0.0825	5.2	18.3	6.6	14.1
	b	**+0.4848** ±0.0781	6.2	23.5	7.5	13.8
Pulse	a	**+0.7142** ±0.0495	14.4	51.0	13.9	13.7
	b	**+0.7349** ±0.0469	15.7	54.0	14.3	13.4

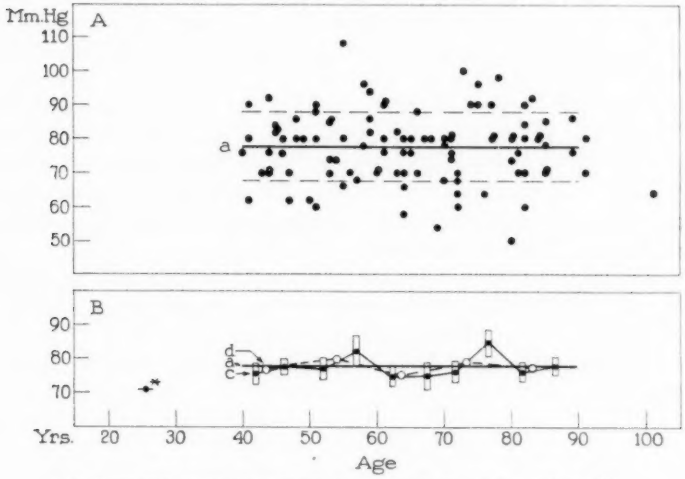
rise from 65 to 89 years. After 89 years, though the observations are few, there is apparently a fall (figs. 1, 3). The usual linear expression (a) ($Y = a + bX$) compared (fig. 3) with a curved one (b) constructed by smoothing the averages of successive five year groups, is lower in the younger age groups, higher in the middle decades, and lower again in the later ones. The straight one (a) is obviously not the curve of best fit. The line (c) which joins the means exhibits inflections suggestive of an S-shaped curve (hyperbole). If a curve (d) is calculated from the formula of a hyperbole,

$$Y = \frac{1}{a + bX}$$

it gives the equation

$$Y = \frac{1}{0.01215 - 0.00007028X}$$

in which Y = systolic blood pressure in millimeter mercury, and X = age in years. But this curve (d) fits very little better than the straight line (a) (fig. 1, table 2). Curves of better fit and eventually the one that fits best may be secured by the use of more complicated polynomial equations. But the relationship (age to systolic pressure), the subject of present interest, may be shown[2] with sufficiently reasonable accuracy, by the smoothed curve (b) of the averages (figs. 1, 3), and the laborious computation of further mathematical analysis is so avoided.



* Average in normal men, aged 21 to 31 years (Addis [5])

Fig. 4. Diastolic blood pressure in normal men over 40 years of age.

A. ●, individual observation. *a*, straight line regression, $Y = a + bX$, with standard error of estimate ---------- for men aged 40 to 89 years.

B. *a*, as under A. *c*, mean of 5 year classes, ± standard error of mean of age and of diastolic pressure. *d*, mean of 10 year classes.

There is mathematical (table 2) as well as graphic evidence that the relation of age to systolic pressure in this sample of men over 40 is represented more accurately by a changing, or curvilinear (b), than by a uniform (a), or linear, relation. The smoothed curve of the five year means presents, as has been said, higher degrees of correlation and of determination and a smaller standard error of estimate. By such an analysis, 39.9 per cent of the "variance"[3] in systolic pressure is accounted for by variance

[2] See Ezekiel—Methods of correlation analysis, Chapter 8.

[3] "Variance" means Standard Deviation squared.

in age in years; by the calculated hyperbole 34.4 per cent; by the linear correlation 34.9 per cent.

The variance is greater in the older men than in the younger; both the standard deviation and the standard error of estimate increased with age. In the smoothed curve, the latter increased from 10.3 mm. Hg in the fifth decade to 23.5 mm. in the ninth decade (fig. 1). It was 15.4 mm. in the first twenty-five year group (40 to 64 years) and 20.5 mm. in the second (64 to 89 years).

2. *The diastolic pressure.* (a) The average diastolic pressure varied slightly in succeeding decades. The mean value in the 5 year groups ranged from 75 mm. Hg (at 65–69 and 70–74 years) to 85 mm. (80–84 years); in the 10 year groups it varied between 75 and 80 mm. (table 1, fig. 4 B).

The extent of scatter was approximately the same at all ages; it was smallest in the period 40 to 49 years (fig. 4 A). The lowest diastolic pressure was 50 mm. in a man of 80 years, the highest 108 mm. at 55 years. In the latter case there may be an error; the pressure in his left arm was 90 mm. The next highest diastolic pressure was 100 mm. in a man of 73 years; in his left arm it was 86 mm.

The standard deviation, ranged from 6.4 mm. (8.2 per cent) to 12.8 mm. (15.5 per cent). The standard deviation was smaller at ages 45 to 49, and 50 to 54 years than in other groups (table 1).

(b) The relation to age follows an uninflected horizontal line (fig. 4 B). The equation is

$$\overline{DP} = 77.2 + 0.007 \times A,$$

where $\overline{DP}$ = diastolic pressure in millimeters of mercury and
A = age in years.

When $A = 40$, $\overline{DP} = 77.49$;
$A = 90$, $\overline{DP} = 77.85$.

The coefficient of correlation is $+0.0097 \pm 0.101$ (tables 1, 2).

The correlation for the entire series of 103 men is likewise not significant, -0.0346 ± 0.0994. The equation is then

$$\overline{DP} = 79.1 - 0.024 \times A$$

When $A = 40$, $\overline{DP} = 78.1$;
$A = 90$, $\overline{DP} = 77.0$;
$A = 100$, $\overline{DP} = 76.7$.

(c) The standard error of estimate is 10.3 mm. Hg.

3. *The mean pressure.* The mean blood pressure is the level midway between the systolic and diastolic levels.

a. The average mean pressure in succeeding 5 and 10 year classes presented an increase with age (table 1, fig. 5 A and B). The rise was similar to that of the systolic pressure, but modified by the constancy of the diastolic pressure. The average mean pressure in those aged 40 to 44 years was 96 mm. A slight rise occurred up to 55 years, a more marked rise after 75 years (fig. 5 B). The individual scatter ranged from 79 mm. in a man of 57 years to 140 mm. in one man of 73 years and in another of 85 years. The scatter was more marked in the later decades (fig. 5 A).

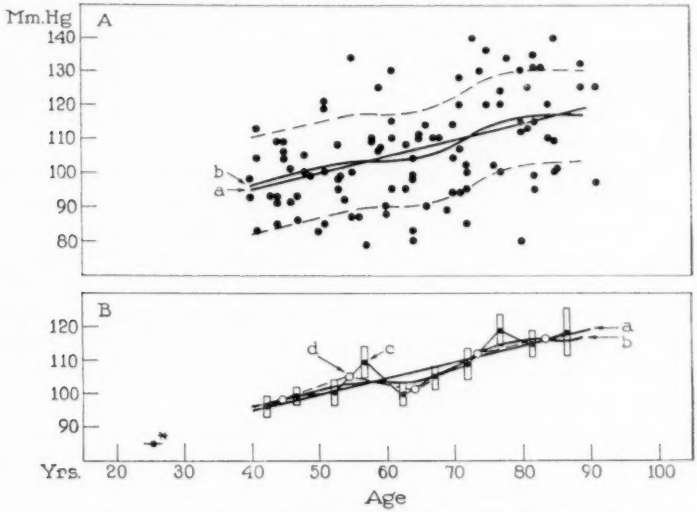


Fig. 5. Mean blood pressure in normal men over 40 years of age under basal conditions.

A. ●, individual observation. *a*, straight line regression, $Y = a + bX$. *b*, curvilinear regression, smoothed curve of averages of 5 year classes, with standard error of estimate ---------- for men aged 40 to 89 years.

B. *a*, as under A. *b*, as under *A*. *c*, average of 5 year classes, ± standard error of mean of age and of mean pressure. *d*, average of 10 year classes.

The standard deviation in the 5 year groups varied from 7.0 to 16.2 mm., the coefficient of variation from 7 to 16 per cent. The deviation was smallest in the fifth decade.

b. The linear equation for the relationship, from 40 to 89 years, is:

$$\overline{MP} = 76 + 0.477 \times A$$

where MP = mean blood pressure in millimeters of mercury and
A = age in years.

When $A = 40$, $\overline{MP} = 95$;
$A = 90$, $\overline{MP} = 119$.

The coefficient of correlation, $+0.4279 \pm 0.0825$, is highly significant (tables 1, 2). If the results of the entire series of 103 men (to 101 years) are analyzed, the slope of the regression and the coefficient of correlation are approximately the same as they are in the series of 100 (to 89 years).

c. Though the straight line presents a highly significant positive correlation with age, inspection of the averages for each 5 and 10 year class indicates that an inflected curve is more accurately descriptive (fig. 5 A). A curve obtained by smoothing[4] the five year averages has a higher index of correlation, a higher index of determination and a smaller standard error of estimate (table 2). It presents a gradual rise of the mean blood pressure from 96 mm. at age 40, to 104 mm. at age 57; a constant level to age 62; then a rise to 116 mm. at age 81.

4. *The pulse pressure.* In view of the rise of the systolic pressure and the constancy of the diastolic level with increase of age, it is obvious that the pulse pressure should follow the trend of the systolic pressure.

a. The average of the 5 year groups increased gradually (table 1, fig. 6 B) from 40 mm. Hg (40 to 44 years) to 49 mm. (60 to 64 years). Thereafter the rise was greater reaching 82 mm. (85 to 89 years) and finally declined (three subjects over 90 years). The standard deviation varied from 5.8 mm. (40 to 44 years) to 21.5 mm. (85 to 89 years) (table 1). The deviations were smaller in the younger age groups.

The means of the 10 year classes showed similar changes.

b. The linear relation of pulse pressure to age from 40 to 89 years is given by the equation:

$$\overline{PP} = 1.002 \times A - 7.307$$

where $\overline{PP}$ = pulse pressure in millimeters of mercury and
A = age in years.
When $A = 40$, $\overline{PP} = 33$;
$A = 90$, $\overline{PP} = 83$.

The increase is 10 mm. per decade. The coefficient of correlation, $+0.7142 \pm 0.0495$, is highly significant (tables 1, 2).

If the results of the series of 103 men are calculated, the relations are essentially similar.

c. As with the systolic and the mean pressures, the relation of the pulse pressure to age is represented more accurately by a curvilinear regression with significant inflections beginning at the 60th year (fig. 6). The curve, obtained by smoothing the 5 year averages, has a higher degree of correlation, a higher index of determination, and a smaller standard error of

[4] The adequacy of the smoothed curve has been discussed in the section on the systolic pressure.

estimate than the straight line (table 2). According to this curve the pulse pressure rose from 40 mm. (age 40) to 49 mm. (age 62) and then to 81 mm. (age 85) (fig. 6). In keeping with the wide range of variation in the older men, the standard error of estimate increased with age, from 6.7 mm. Hg (the fifth decade) to 17.9 mm. (the ninth decade). In the men aged 40 to

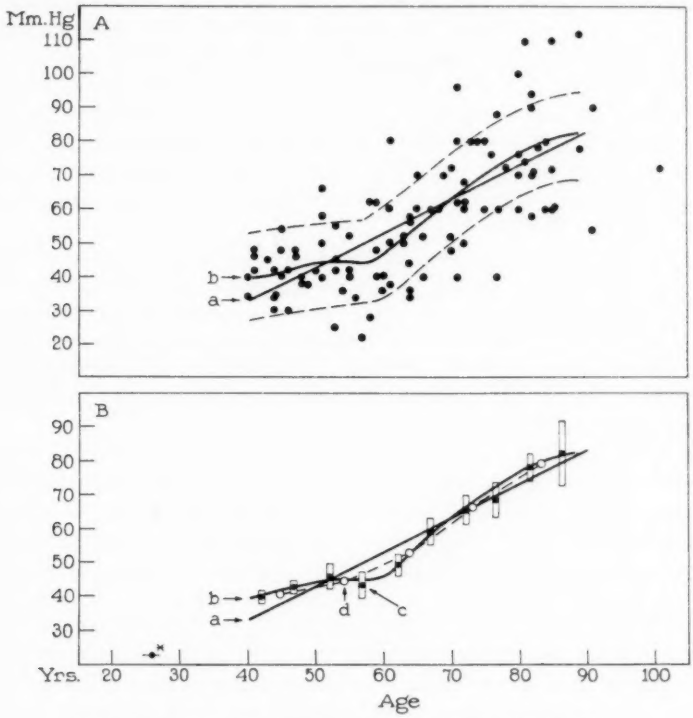


Fig. 6. Pulse pressure in normal men over 40 years of age under basal conditions.

A. ●, individual observation. *a*, straight line regression, $Y = a + bX$. *b*, curvilinear regression, smoothed curve of means of 5 year classes with standard error of estimate ---------- for men aged 40 to 89 years.

B. *a*, as under A. *b*, as under A. *c*, average of 5 year classes, ± standard error of mean of age and of pulse pressure. *d*, average of 10 year classes.

64 years, it was 10.7 mm.; in those aged 65 to 89 years, 15.4 mm. These figures are included in the tables.

Discussion. Reports on the blood pressures, including variation with age, sex, and other factors, have been numerous, as has been mentioned. Measurements published by insurance companies have gained authority

beyond other reports because of the large number of observations on which they are based, and have influenced current views of what is considered normal. But conceptions of what is normal are dependent on definitions of normality. The conditions under which blood pressures are taken obviously influence their level profoundly. Vast accumulations of data do not assure correctness of results, especially when reliance has been placed on a multiplicity of observers who may have been unequally trained, when the conditions of observation have been inadequately and inexactly defined, and when the selection of subjects has not been sufficiently random. After age 60 years, the reports have been based, furthermore, on the result of pooling all measurements indiscriminately. The present analysis suggests that this practice does not permit the facts to emerge. For soon after the 60th year, inflections have appeared in the curves now presented.

It has, for good and obvious reasons, been customary to make observations during daytime activity,[5] when the pressures, as is well known, are elevated (Addis (5), table 3). Many such observations have been recorded (4, 6, 7, 8, 9, 10, 11, 12). The value of securing measurements at low levels of activity under basal conditions has been mentioned by several investigators (5, 13, 14). It becomes even more apparent in the present and in related, further studies.

The *systolic pressure* in normal men at the age of 29 years is usually given as 115 mm.; at 30 years, 120 mm.; at 40 years, 125 mm.; at 50 years, 130 mm.; and at 60 years, 135 mm. (15). When it is above 150 mm. Hg under conditions of daily activity it has generally been considered abnormal at all ages (4, 9, 12, 16, 17). In the opinion of Alvarez and Stanley (1930) a pressure of 115 mm. is just as normal and one of 140 mm. just as abnormal in an old man as in a young man; there is no difference between the two. But they regarded the mode (the most frequent pressure) to be more significant than the mean. It is necessary to insist that in their study, only 58 men were aged 60 to 84 years.[6] Although the modal pressure of this age class did not rise, the mean was higher than in the younger groups. Symonds likewise separated the pressures of a large number of insurance applicants into 5 year classes, but he did so only to 59 years. After 60 years he included all cases in one class. That there is value in emphasizing the need of studying men in older groups separately

[5] "Daytime activity" includes occupations and situations of great diversity, physical and psychological. What is not provided is uniformity. In private medical practice, daytime activity represents examination under conditions usual in an office (4). Similar remarks may be made of public practice. But these conditions in themselves are not usual. Addis (5) examined men who had been sitting 10 to 15 minutes, during an interval when they were excused from the daily activities of a military camp.

[6] There were 3677 white prisoners, within 10 per cent of normal weight.

in 5 year classes emerges from the observations of Bowes (6), Norris (8), Thompson and Todd (18), Morlock and Horton (19), who noticed an elevation of the systolic pressure to 160 mm. and above in men over 70 years.

From this study emerges, what seems a fact, that the mean and modal systolic pressures increase with age and do so in a more marked degree, after the 65th year.

Diastolic pressure. The constant level of the diastolic pressure found in this study is in agreement with the general view that its level is unaffected,

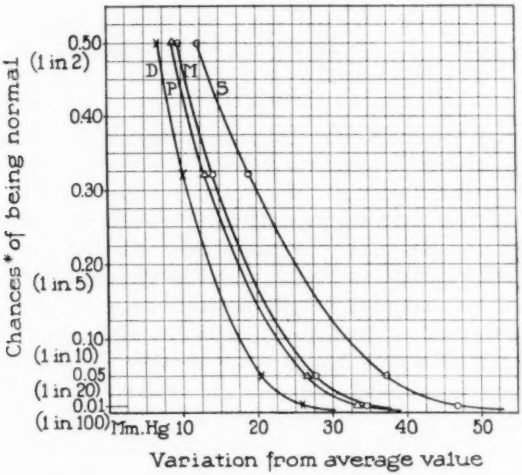


Fig. 7. Curves showing the probability that the observed blood pressures are normal, expressed as variations from the average value in men over 40 years of age. *S*, systolic pressure; *D*, diastolic pressure; *M*, mean pressure; *P*, pulse pressure.

* The phrase used for this expression by Ezekiel (20) is the "probability of specified departure" from the average value.

Note: When in an individual the systolic pressure differs 47 mm., the diastolic pressure 26 mm., the mean pressure 35 mm., or the pulse pressure 34 mm., from the respective average, the chances are only 1 in 100 that the measurement is normal.

unlike that of the systolic pressure, by age. It is between 60 and 90 mm. in adults (15), and between 80 and 90 mm. in older men (6, 8, 9, 12, 18).

Mean blood pressure. Since the diastolic pressure remains constant the influence of the systolic level upon the mean results in a course similar to that of the systolic pressure.

Pulse pressure. In normal individuals the pulse pressure has been placed between 30 and 50 mm.[7] (5). Below 20 mm. and above 50 mm. is regarded as abnormal.

[7] Instead of 50 mm. White (15) gives the figure 60 mm.

In the reports of Symonds (9), and of Alvarez and Stanley (12), there could be no inflections with age in view of the fairly constant systolic and constant diastolic levels found by them. Bowes (6) and Thompson and Todd (18) give an increase above 50 mm. in men over 70 years. In his carefully controlled study of young men 21 to 31 years old, Addis (5) observed an average level of 28 mm. under basal conditions and of 50 mm. during daytime activity. In the present investigation the regression curve rose to a level above 50 mm. after the 67th year, and above 65 mm. after the 70th year. The mode also increased with age but is not shown in the figure.

The chances that an observed blood pressure is normal may be predicted according to the deviation from the average (fig. 7).

SUMMARY

1. The blood pressures have been measured, under basal conditions, in 100 normal men, 20 in each decade, from 40 to 89 years, in two men of 91 and one man of 101 years.

2. The data have been statistically analyzed and the various measures and graphs of correlation and variation presented.

3. The systolic, mean, and pulse pressures showed a highly significant positive relation to age, but not the diastolic pressure.

4. The average values for the systolic, mean, and pulse pressures of the men, grouped in succeeding half or whole decades, indicated that the relation of these pressures to age changes and is therefore curvilinear, rather than uniform and linear. Three curves of the systolic pressure (a) the straight line, $Y = a + bX$, (b) the S-curve of the 5 year averages, smoothed mathematically, (d) the hyperbolic curve, $Y = \frac{1}{a + b\,X}$, were analyzed; all presented highly significant relationships. The smoothed curve was the best fit.

5. The systolic, mean, and pulse pressures increased gradually from 40 to 62 years and rapidly from 62 to 85 years. After 90 years, the pressures seem to decline; but the number of observations is too few to render an inference valuable.

6. The mode of the systolic, of the mean, and of the pulse pressures of men, arranged by decades, increased with age. The mode was sharply concentrated in the younger men.

7. The studies of other observers have been discussed. The results of this study are at variance with the observations of several other investigators because, among other reasons, after 60 years of age significant inflections occur which are lacking in theirs due to a lack of detail in their analyses.

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THE EFFECT OF DESTRUCTION OF THE SPINAL CORD ON HYPERTENSION ARTIFICIALLY PRODUCED IN DOGS[1]

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In the course of a series of experiments, the purpose of which has been to determine, if possible, the various factors concerned in the development of essential hypertension, the spinal cords of a small series of dogs in which hypertension had been produced by the method of Goldblatt, were destroyed below a certain level. It is the purpose of this report to record the effects of destruction of the cord on five such hypertensive animals.

METHOD. After the satisfactory production of a Van Leersum carotid loop, careful daily blood pressure observations were made over a period of two weeks or more to determine the average normal blood pressure of the animal. Hypertension then was produced by the application of Goldblatt clamps to the renal arteries. Again daily blood pressure observations were made for one month or more for two purposes,—to indicate the permanency of the hypertension produced and to determine the average blood pressure of the now hypertensive animal.

If after a month the elevated blood pressure in these animals showed no tendency to fall, a laminectomy was performed in the low cervical region; the spinal cord was sectioned and the portion below the section destroyed with a soft solder rod. After this operation the animals were nursed with the greatest care and daily blood pressure observations made until they were sacrificed. The various surgical procedures in the course of the experiments invariably were performed under complete anesthesia.

Effects of destruction of the spinal cord in hypertensive animals. On the five animals subjected to this experiment, the effects were similar. The destruction of the cord was followed immediately by a sharp fall in blood pressure *well* below the previous average normal blood pressure of the animal. Following this preliminary fall there was a gradual rise in blood pressure to a level above the previous average normal blood pressure. In two animals observed 40 and 60 days after the destruction of the cord, the

[1] Done under a grant from the John and Mary Markle Foundation.

blood pressure approached at times, but never reached or maintained the hypertensive level. The protocols of the five experiments are as follows:

Experiment 1. (Dog SA-36-21): Normal blood pressure 140 mm. Hg. Constriction of renal arteries; rise of blood pressure to 250 mm. Hg with maintenance at a level of 190 mm. Hg for 31 days. Laminectomy and destruction of the cord; sharp fall of blood pressure to 100 mm. Hg; gradual rise of blood pressure reaching 170 mm. Hg in 25 days and maintained at 130–150 mm. Hg until animal was sacrificed 61 days after destruction of the cord.

Experiment 2. (Dog SA-36-31): Normal blood pressure 120 mm. Hg. Constriction of renal arteries; rise of blood pressure to 260 mm. Hg with maintenance at a level of 220 mm. Hg for 39 days. Laminectomy and destruction of cord; sharp fall of blood pressure to 90 mm. Hg; a gradual rise of blood pressure reaching a maximum of 210 mm. Hg and maintained at 160 mm. Hg until a few days before the animal died when it fell to 130 mm. Hg.

Experiment 3. (Dog SA-36-9): Normal blood pressure 118 mm. Hg. Constriction of renal arteries; rise of blood pressure to 208 mm. Hg within 3 days with maintenance at a level of 180 mm. Hg for 35 days. Laminectomy and destruction of cord; sharp fall of blood pressure to 92 mm. Hg. Two days later the pressure had risen to 100 mm. Hg. The animal died on this day from an intracystic hemorrhage.

Experiment 4. (Dog SA-36-81): Normal blood pressure 160 mm. Hg. Constriction of renal arteries; rise of blood pressure to a maximum of 270 mm. Hg with maintenance at a level of 250 mm. Hg for 76 days. Laminectomy and destruction of cord; sharp fall of blood pressure to 120 mm. Hg. Gradual rise of blood pressure to a maximum level of 250 mm. Hg with maintenance at over 220 mm. Hg. The animal was sacrificed 45 days after the destruction of the cord.

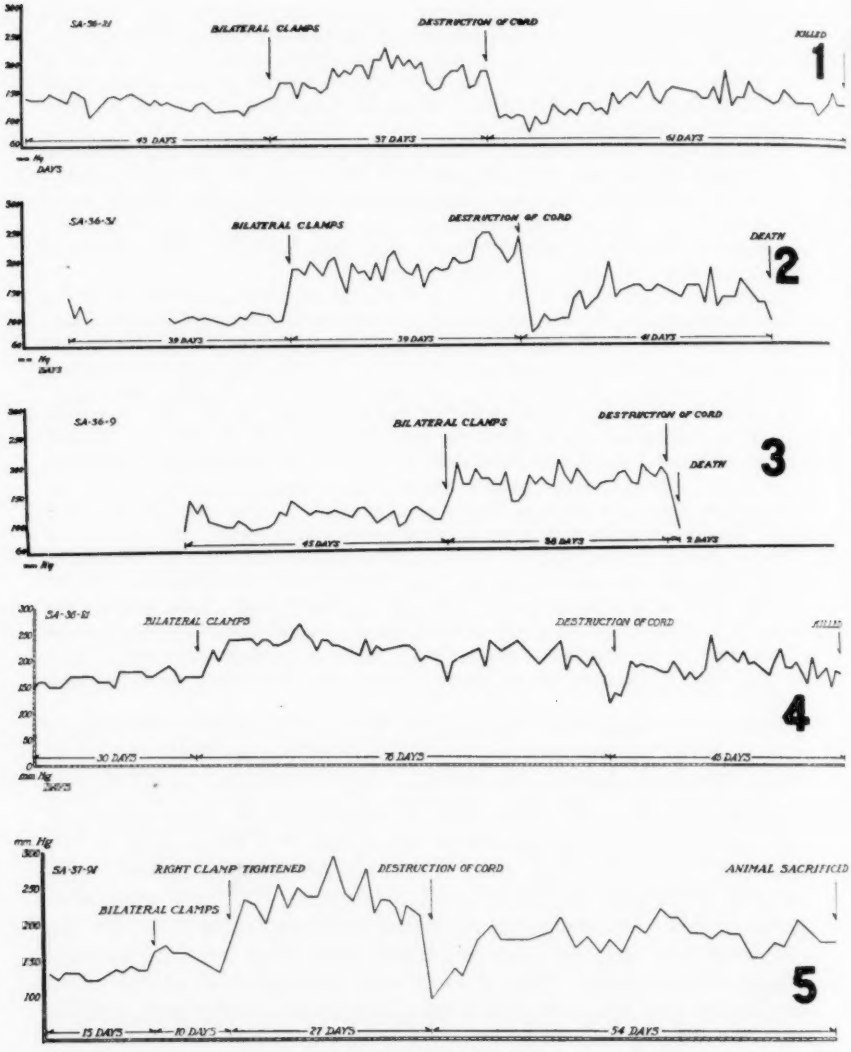
Experiment 5. (Dog SA-37-91): Normal blood pressure 130 mm. Hg. Constriction of renal arteries; transient elevation of blood pressure to a maximum of 285 mm. Hg with maintenance at a level of 250 mm. Hg for 27 days. Laminectomy and destruction of the cord; sharp fall of blood pressure to 95 mm. Hg. Gradual rise of blood pressure to a maximum of 220 mm. Hg with maintenance at a level of nearly 200 mm. Hg. The animal was sacrificed 54 days after the destruction of the cord.

Summary of clinical and pathological findings. To avoid repetition the findings of the various experimental animals are summarized.

The clinical findings and course were such as to leave no doubt that there had been complete destruction of the cord below the ligature (C_5).

Spinal cord. The appearance of the spinal cord at autopsy in the animals subjected to destruction in the manner described, varied with the time which had elapsed after the procedure. Within a week after operation, the vertebral canal contained extensive blood clots and the cord itself was softened and extensively infiltrated with hemorrhage throughout its extent. After 6 weeks the hemorrhage in the vertebral canal had largely disappeared, but the spinal cord showed definite destruction. Sections of the cord were taken at various levels (C_1, C_5, D_1, L_1, etc.) and fixed in Mueller's fluid and stained with osmic acid; other sections were stained with hematoxylin and eosin. The sections showed marked edema, destruction and disappearance of the nerve cells of the

anterior and posterior horns and extensive degeneration of the fiber tracts. For the most part, the sections show complete anatomical destruction



Experiment 1 to 5

of the cord and so extensive are the changes that there can be little doubt of its complete loss of function.

Heart. The cardiac muscles of the five animals show mild cardiac hypertrophy.

Kidneys. The kidneys show some pathological changes with evidence of a glomerular nephritis. Some vascular changes also are evident, especially in the arterioles. A small amount of cellular exudate may justify the diagnosis of interstitial nephritis.

Liver and pancreas. There are no significant changes in these organs.

The effect of destruction of the spinal cord upon the blood pressure level in animals with experimental hypertension

No.	Normal blood pressure	Constriction of renal arteries	Destruction of spinal cord	Re-elevation to hypertensive level
I	140 mm. Hg	250	100	61 days → 170
II	120 mm. Hg	260	90	41 days → 210
III	118 mm. Hg	208	92	2 days → 100 †
IV	160 mm. Hg	270	120	45 days → 250
V	130 mm. Hg	285	95	54 days → 220

Fig. 1

Spleen. There is a definite thickening in the walls of the arteries and arterioles associated with diminished lumen.

Discussion. Von Bezold showed that in curarized rabbits, electrical stimulation of isolated segments of the spinal cord resulted in a rise in the blood pressure, the extent of the rise varying with the segment stimulated. Stimulation of the cervical segments was without effect but below the third thoracic segment, the arterial pressure was markedly increased. Bradford found that stimulation of the anterior nerve roots within the

dura of dogs, from approximately the 6th dorsal to the second lumbar segments, caused a marked elevation of the blood pressure. Stimulation of the 10th to 13th dorsal roots was especially effective. Division of the anterior nerve roots from the 6th dorsal to the second lumbar in patients with hypertension, causes a fall in blood pressure, the reduction in some cases continuing over a period of 3 years. These observations indicate that the splanchnic vessels constitute an important flexible reservoir which governs the level of arterial pressure and suggests that in essential hypertension the elevated blood pressure may be due in part to the constriction or loss of elasticity (or both) of the splanchnic vessels. The question is, how extensive or effective is the control of these vessels by the central nervous system.

The experiments herein described were undertaken with the object of determining, if possible, the relationship between the central nervous system and experimental hypertension. On the basis of experimental and clinical evidence, it would seem that the destruction of the spinal cord below the 5th cervical should eliminate the part of the central nervous system which is chiefly concerned with the maintenance of the level of blood pressure and, presumably, with hypertension. The experiments show that in dogs with established hypertension, the destruction of the spinal cord below the level of the 5th cervical vertebra immediately is followed by a sharp fall in blood pressure; subsequently, however, the pressure again rises to a level exceeding that recorded as normal for the animal before hypertension was produced. During the period of observation after destruction of the cord, the blood pressure in each animal failed to reach the maximum hypertensive level previously produced and tended to fall toward the termination of the experiment.

SUMMARY

The spinal cord has been destroyed in each of five dogs with an hypertension produced by partial constriction of both renal arteries. These experiments have shown that destruction of the cord below the 5th cervical vertebra produces an immediate sharp fall in the systolic blood pressure, which is followed by a rise to above the normal, but never to the hypertensive level.

THE EFFECT OF RATE OF HEATING AND ENVIRONMENTAL TEMPERATURE ON PANTING THRESHOLD TEMPERATURES OF NORMAL DOGS HEATED BY DIATHERMY

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There are two theories concerning the origin of thermal tachypnea, or panting, of dogs. According to the older theory, first proposed by Goldstein, panting is caused by the heated blood stimulating thermosensitive regions of the brain. This theory is based on experiments such as those of Kahn (1) and Heymans and Heymans (2) wherein the carotid artery was heated by cuff heaters through whose hollow walls warm water was circulated. Jelsma (3) localized the site of the thermosensitive portion of the brain to the fore- and mid-brain by ligating the basilar artery before heating the carotid blood. Such a theory demands an increase in body temperature, i.e., blood temperature, before "central" panting can be induced. The second theory was first proposed by Sihler (4) who noted that in intense sunlight dogs could pant without an increase in rectal temperature. This "reflex" panting is presumably caused by a stimulation of the warm receptors of the skin. Richet (5) in his dual theory claimed that both mechanisms were active but that central panting did not commence until the blood temperature reached 40.7°. By the use of chloralose anesthesia, peripheral panting could be suppressed and central panting alone remained, although Richet did not consider a possible reduction of central sensitivity as a result of anesthesia which was shown later by Nikolaides and Dontas (6).

In the development and elaboration of these theories concerning the origin of panting no measurements have been made of skin temperature although the reflex theory is based on such changes. Furthermore, the earlier work has been done largely on anesthetized animals. In such experiments all anesthetics to our knowledge produce either a variable amount of central depression or peripheral vasomotor disturbances, both of which affect the temperature regulating mechanism. It seemed worthwhile, therefore, to make a study of the skin and body temperature thresh-

[1] Sterling Fellow, Yale University. On leave of absence from the University of Minnesota 1936–37.

olds at which panting occurred, using the following improvements over the older technique: 1, the use of trained unanesthetized dogs; 2, a 150 minute rest to obtain thermal equilibrium before heating; 3, controlled rate of heating by the use of a high frequency diathermy machine, and 4, a controlled thermal environment. The skin and body temperatures, both basal and threshold for panting, were measured in two series of experiments, namely, *a*, the first series in which the rate of heating was varied in a medium environment, and *b*, the second series in which the rate of heating was kept constant and equal to the b.m.r. while the environmental temperature was varied from that of a cool environment to a warm environment but kept constant for each experiment.

METHODS AND PROCEDURE. Dogs were trained to lie on a table for five to six hours. A thin sheet of tin was placed beneath the dog for one diathermy electrode and the other electrode was attached to the upper flank of the recumbent dog by a harness. Temperatures were measured by copper constantin thermocouples at eleven places in the skin. The rectal temperature was measured by a thermocouple six inches in the rectum. The thermocouples of light construction were sealed to the skin by adhesive tape of a size of an inch by a quarter of an inch. The thermocouples were placed at the following places: head between the ears, foreleg, shoulder, thorax, back, ear, upper flank, lower flank, lower abdomen, lower thorax, lower shoulder. The animals were rested two and a half hours to obtain basal conditions. The temperatures obtained after this rest are called basal temperatures. In the earlier observations respiration rate was recorded by a string stretched across the thorax and attached to a kymograph lever. The onset of panting could readily be ascertained by 1, the abrupt increase in rate; 2, the breathing through the opened mouth; 3, a yawning which precedes panting. In the later experiments the kymograph recording was found to be unnecessary. About one to two minutes after panting had started the diathermy machine was turned off and the temperatures again measured. These were the panting temperatures.

The diathermy machine used has been described by Hemingway and Witts (7).[2] It generates a high frequency current of one million cycles per second, and with this frequency the rate of heating is simply the product of high frequency voltage times high frequency current since the phase angle is zero, as shown by Hemingway and McClendon (8). The voltage and current were measured by thermocouple meters. The wattage output of the machine was adjusted by varying a tuning condenser in the output circuit which contained the dog. The thermocouple system for temperature measurement has been described by Clark (9).

RESULTS AND DISCUSSION. *Time relations of skin temperature and respiration rate.* Figure 1 shows how some of the skin temperatures vary

[2] Built with the aid of a grant from the American Medical Association.

during the course of a treatment. These results are taken from one of the preliminary experiments in which the diathermy current was interrupted every ten minutes for temperature measurements. Only rectal, head, ear and electrode temperatures are given, these being typical. During the panting threshold temperature measurements the diathermy current was on continuously until panting occurred.

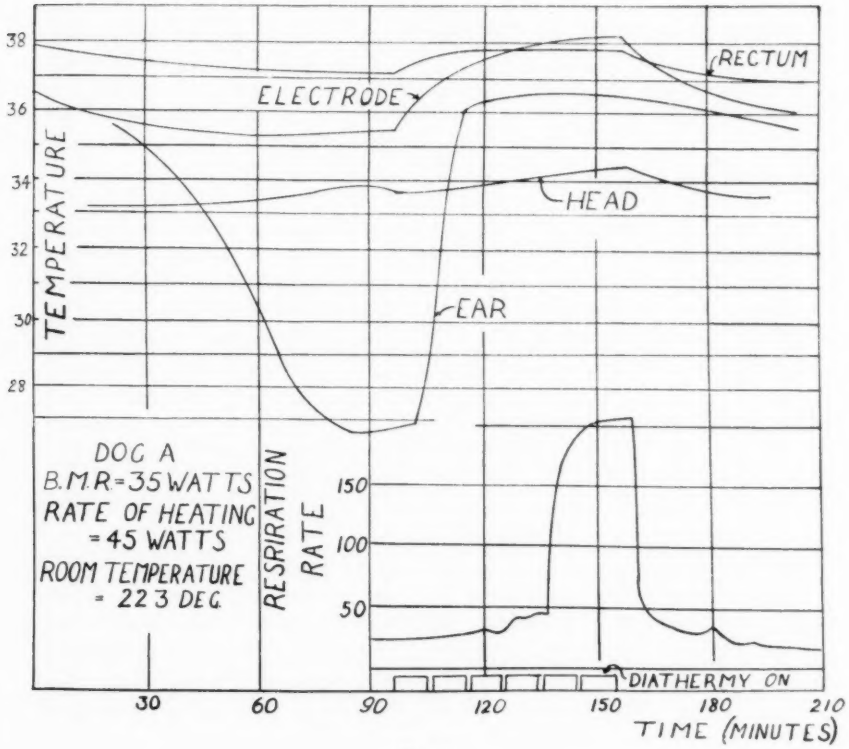

Fig. 1

The ears of dogs as temperature regulators. Of special interest are the temperature changes of the ears. After a two and a half hour rest in a cool environment, 20 to 23°, the ear temperature will decrease to a low value of 25 to 27°, this temperature being the lowest of the skin temperatures. After heating with diathermy there is first a period of continued lower ear temperature followed by a sudden rise of temperature to within two degrees of the rectal. The ear temperature is now the highest of the exposed skin temperatures. These can also be seen in the last four columns of tables

1 and 2 where ear and head temperatures are given. This remarkable lability of ear temperatures indicates that the ears of dogs are of special

TABLE 1

Variation of rate of heating

Environmental temperature 26–27°

1	2	3	4	5	6	7	8	9	10	11	12	13
RELATIVE HUMIDITY	HEAT DOSAGE B.M.R.	CALORIES	BASAL RECTAL TEMPERATURE	PANTING RECTAL TEMPERATURE	$\Delta T/\Delta t$ RECTAL	BASAL SKIN TEMPERATURE	PANTING SKIN TEMPERATURE	$\Delta T/\Delta t$ SKIN	EAR TEMPERATURE BASAL	EAR TEMPERATURE PANTING	HEAD TEMPERATURE BASAL	HEAD TEMPERATURE PANTING
Dog A. B.M.R. = 35.0 watts												
					deg./hour			*deg./min.*				
35	0.5	13.2	37.6	37.9	0.4	34.8	35.4	0.58	34.8	36.5	35.2	36.6
44	0.5	10.1	37.8	37.8	0	34.8	35.5	1.09	28.7	36.6	34.7	35.3
15	0.75	8.2	37.4	37.5	0.4	34.8	35.2	1.12	34.3	36.7	34.7	35.0
35	0.75	8.2	37.6	37.7	0.3	35.2	35.5	0.80	35.2	36.5	35.0	35.0
33	1.0	8.9	37.7	37.9	0.8	34.6	35.4	2.82	28.4	36.6	35.0	35.7
26	1.0	9.4	37.4	37.6	0.7	34.5	35.2	2.23	28.5	36.5	34.8	35.1
20	1.25	10.1	37.1	37.5	1.5	34.5	35.1	2.25	34.2	36.5	35.0	35.2
25	1.25	6.8	37.5	37.6	0.3	34.9	35.3	2.02	34.7	36.4	35.2	35.5
24	1.5	8.9	37.4	37.6	1.0	34.7	35.4	3.25	31.3	36.5	34.8	35.1
17	1.5	10.8	37.4	37.8	1.6	34.5	35.1	2.36	28.3	36.3	35.3	35.4
31	1.75	4.5	37.3	37.4	1.2	35.0	35.2	2.64	36.5	36.5	34.8	35.1
21	1.75	4.9	37.4	37.6	3.0	34.6	35.1	3.75	32.0	36.2	34.8	34.9
Dog B. B.M.R. = 38.7 watts												
33	0.5	10.3	37.8	38.1	0.5	35.3	35.8	0.85	30.5	35.7	35.3	35.7
34	0.5	15.9	38.0	38.3	0.3	35.5	36.2	0.81	29.2	36.5	35.4	35.6
35	1.0	8.6	37.7	38.1	1.4	35.4	36.1	2.26	30.0	35.9	34.4	35.4
43	1.0	15.9	37.7	38.1	0.8	35.3	36.0	1.45	29.5	36.4	35.3	35.4
30	1.0	16.8	37.7	38.0	0.6	35.0	35.9	1.84	29.5	37.0	35.5	35.5
25	1.5	12.1	37.7	38.0	1.1	35.9	36.3	1.20	36.3	36.9	35.7	36.4
36	1.5	16.3	37.8	38.4	1.8	35.4	36.3	2.92	34.1	37.4	35.2	35.6
Dog C. B.M.R. = 47.1 watts												
38	0.5	9.0	37.7	38.0	0.8	34.3	34.9	1.45	29.8	32.6	35.3	35.6
34	0.5	7.7	37.7	38.0	0.8	34.5	35.0	1.25	29.4	32.4	35.3	35.3
29	0.75	16.2	37.6	38.0	0.8	34.4	35.4	1.94	29.4	36.4	34.8	35.2
28	1.0	18.0	37.4	38.0	1.4	34.0	35.6	3.60	29.3	35.8	34.0	35.3
30	1.0	14.2	37.3	38.1	2.3	34.0	34.5	1.49	29.5	30.3	35.1	35.2
40	1.5	8.1	37.1	37.4	2.2	34.1	34.9	5.92	29.4	36.3	34.7	35.0
39	1.5	7.6	37.1	37.6	3.8	34.1	34.5	3.43	30.7	30.9	35.0	35.7
30	1.5	11.2	37.6	38.0	2.2	34.5	35.6	5.83	31.0	36.5	35.7	35.8

importance in temperature regulation. The ears of dogs usually possess a large surface and are out of proportion for purposes of audition. The

ability to be cooled or warmed, the large radiating and conducting area all indicate that the ears of dogs function as large radiating surfaces in the regulation against heat.

The "first and second line of defense" theory. Cannon, Querido, Britton and Bright (10) in their study of the part played by epinephrine and the sympathetic nervous system in body temperature regulation against cold have proposed a "first and second line of defense" theory for chemical heat regulation in which sympathetic activity is the first line of defense against cold and shivering is the second line. In the physical regulation against heat, if we use Cannon's analogy, there are also two lines of defense. The "first line of defense" is a peripheral vasodilatation in which the ears play a major rôle. This is accompanied by an increased blood flow and dilution of the blood, as shown by Lozinsky (11). The second line of defense is panting which is evoked when a greater heat loss is required.

The induction of panting by diathermy in an environment of 20 to 30° differs from previous methods. In the older methods animals have been placed in a warm environment and their body temperature has been raised by reduction of heat loss. In an environment where the temperature approaches that of body temperature there is relatively less heat lost by radiation and more by evaporation as shown by Rubner (12). The first line of defense, i.e., peripheral vasodilatation, increases the heat loss by radiation and conduction, and these processes become ineffective in a warm environment. Hence when panting is caused by placing dogs in a warm environment the onset of peripheral vasodilatation, as indicated by a rise in ear temperature, and panting are likely to be almost simultaneous. The method used in our experiments is unique in allowing a considerable heat loss by radiation and conduction as well as by evaporation and is more suitable for enabling a separation to be made of the two lines of defense. In all our observations peripheral vasodilatation always preceded panting.

The skin temperature of the head, neck, shoulder, thorax and back is always well above room temperature, being of the order of 33 to 35°. On heating by diathermy these skin temperatures increase only a slight amount in comparison to the ears. The heat loss from the skin of the head, neck and trunk is not appreciably increased during a mild heat treatment of one b.m.r. dosage. The ear temperature, however, shows the rapid increase indicated in figure 1. This leads to the interesting conclusion that the skin of the head, neck and trunk loses heat at a fairly uniform rate before and during heating, while the fine regulation for which a variable heat loss is required is the function of the ears. An analogy is the method often used in maintaining a hot water bath. One heater is used to operate continuously while a small heater controlled by a thermostat operates intermittently for the fine regulation.

Effects of variation of rate of heating. In table 1 there are given the basal and panting rectal temperatures, the average of the skin temperature

values, the temperatures of the ear and the head, as well as the heat dosage as a fraction of the b.m.r. Thus with dog A, whose b.m.r. was 35.0 watts with a heat dosage of 0.5 b.m.r., the heat dosage was 17.5 watts and the dog was required to dissipate at least 52.5 watts of heat. The values in column 3 marked "Calories" are the number of calories of heat received by the dog to produce panting. The rate of rectal temperature increase expressed as degrees per hour is given in column 6 while a corresponding value for the average skin temperature is given in column 9. The average skin temperature $\overline{T}$ was estimated from the eleven skin temperatures using a formula where

$$\overline{T} = \Sigma AT/\Sigma A$$

A represents approximately the area of the region whose skin temperature is given by T. In this formula the temperatures of the larger areas are weighted according to the magnitude of the area. This method is only approximate but is a more satisfactory criterion than a simple average of the eleven temperatures in which each temperature T would be given equal weight. The room temperature for the experiments of table 1 was maintained between 26 and 27°.

In the series of experiments summarized in table 1, the basal rectal temperatures for each dog fall within a narrow range for the different experiments, the ranges being: A, 37.1–37.8; B, 37.7–38.0; C, 37.1–37.7. The rectal temperatures at which panting starts fall within the ranges: A, 37.4–37.9; B, 38.0–38.4; C, 37.4–38.0. The rectal panting thresholds seem to be independent of rate of heating when this was varied from 0.5 to 1.5 b.m.r. units.

The average skin temperatures under basal conditions at 26 to 27° room temperature were within the ranges as follows: A, 34.5–35.2; B, 35.0–35.9; C, 34.0–34.5. Dog B was thin and emaciated, dog A was intermediate in its nutritive condition while dog C was obese. This observation is similar to that made on humans that obese individuals have cold skins. The threshold average skin temperatures for panting fall within the following ranges: A, 35.1–35.4 (0.3); B, 35.8–36.3 (0.5); C, 34.5–35.6 (1.1). There seems to be no dependence of rectal or skin temperature thresholds on rate of heating as measured by the b.m.r. dosage of heat, nor the rate of increase of the temperatures, $\Delta T/\Delta t$. This is of interest since sensation of warmth does depend on the $\Delta T/\Delta t$ ratio, whereas in our observations, the absolute temperature threshold, while variable, seems more important. It is possible that the range of $\Delta T/\Delta t$ values is not sufficiently large to show an effect on thresholds due to rate. If the rate $\Delta T/\Delta t$ is more effective than the absolute threshold then one would expect to find a low threshold with a high $\Delta T/\Delta t$. This does not occur.

The effect of environmental temperature. In table 2 are given basal and

threshold temperatures when dogs are heated by a heat dosage of 1.0 b.m.r. in environments ranging in temperature from 23 to 28°. After a two and a half hour rest at 23° the thin dog B showed shivering movements while at 28°, the obese dog C was on the verge of panting.

TABLE 2

Variation of environmental temperature

Rate of heating 1.0 B.M.R.

1	2	3	4	5	6	7	8	9	10	11	12	13
ROOM TEMPERATURE	RELATIVE HUMIDITY	CALORIES	BASAL RECTAL TEMPERATURE	PANTING RECTAL TEMPERATURE	RECTAL $\Delta T/\Delta t$	BASAL SKIN TEMPERATURE	PANTING SKIN TEMPERATURE	SKIN $\Delta T/\Delta t$	EAR TEMPERATURE BASAL	EAR TEMPERATURE PANTING	HEAD TEMPERATURE BASAL	HEAD TEMPERATURE PANTING
Dog A. B.M.R. = 35.0 watts												
					deg./min.			*deg./min.*				
23.0	21	18.0	37.2	37.7	1.1	34.2	35.3	2.56	27.0	36.6	33.9	34.1
23.2	20	20.3	37.5	37.8	0.5	33.6	34.7	1.69	25.1	36.2	33.7	34.2
23.9	21	25.7	37.6	37.7	0.1	33.8	35.1	1.61	24.9	36.6	34.4	35.1
24.8	21	18.3	36.7	37.4	1.5	34.4	35.1	1.47	25.2	36.3	34.5	34.7
25.2	28	12.5	37.2	37.6	1.0	34.8	35.2	1.00	35.6	36.6	35.1	35.1
25.4	38	9.3	37.2	37.7	1.8	35.0	35.3	1.07	35.8	36.3	35.1	35.2
28.3	22	4.1	37.6	37.7	0.7	35.8	36.0	1.4	36.6	36.6	35.8	36.0
28.4	10	11.6	37.4	38.1	1.9	35.0	35.6	1.6	35.5	36.7	34.3	34.7
Dog B. B.M.R. = 38.7 watts												
22.9	47	22.8	37.5	38.1	0.9	33.9	35.4	2.3	27.3	36.9	34.2	34.6
23.0	21	21.4	37.6	38.0	0.7	33.8	35.0	1.8	28.0	35.4	34.2	34.8
26.3	35	8.6	37.7	38.1	1.4	35.4	36.1	2.2	30.0	34.9	34.4	35.4
26.5	30	15.9	37.7	38.1	0.8	35.3	36.0	1.4	29.5	36.4	35.3	35.4
28.1	31	10.0	37.6	38.1	1.6	35.9	36.2	1.0	34.3	37.1	35.7	36.0
28.4	29	12.2	37.6	38.0	1.1	36.2	36.7	1.5	32.5	37.3	35.7	36.1
29.2	39	3.9	37.4	37.7	2.1	35.8	36.1	2.1	36.6	36.8	35.0	35.5
Dog C. B.M.R. = 47.1 watts												
23.2	40	30.9	37.8	38.5	0.8	33.3	34.0	0.8	25.9	34.1	34.3	36.5
23.3	45	17.1	37.6	38.1	1.1	33.4	34.5	2.6	27.9	33.7	34.8	34.8
23.4	42	12.0	37.8	38.0	0.9	33.2	34.3	4.0	26.6	35.0	34.5	35.0
26.0	38	14.2	37.3	38.1	2.3	34.0	34.5	1.5	29.5	30.3	35.1	35.2
26.1	30	18.0	37.4	38.0	1.4	34.0	35.6	3.6	29.3	35.8	34.0	35.3
28.1	51	6.4	37.6	37.9	2.2	34.5	35.0	3.2	30.1	34.4	35.1	35.2

The room temperatures have no effect on the basal rectal temperatures nor on the panting rectal temperatures. A low room temperature reduces the average basal skin temperature and also the panting threshold skin temperature. The ranges of panting skin temperatures are: A, 34.7–36.0

(1.3); B, 35.0–36.7 (1.7); C, 34.0–35.6 (1.6). These ranges have been widened due to a wide range of environmental temperatures. From the summarized results in table 2 it is to be noted that the threshold panting skin temperature depends on the basal skin temperature, i.e., a low basal temperature is associated with a low panting temperature. This can be clearly demonstrated by plotting basal temperatures against panting temperatures of table 2. A similar result, although not as clear, is shown by the results in table 1.

Peripheral versus central panting. The question arises in this work whether the panting which results from diathermy heating is caused by the stimulation of skin receptors or by central nervous system receptors. In all cases except one there was a rise in rectal temperature. In many instances this was slight and quite variable, whereas the skin temperature rise was greater and more constant. Using anesthetized dogs both Richet and Anrep and Hammouda found much greater body temperature increases required for central panting than have been observed in tables 1 and 2. For this reason it is believed that the type of panting noted here is peripheral panting caused by the warming of peripheral temperature receptors beneath the electrodes by the diathermy current, and at distant skin areas by blood flow from the heated area facilitated by peripheral vasodilatation most marked in the ears. The central effect is not entirely precluded. When anesthetics are used it is quite possible that the activity of the central receptors have been depressed so that a much greater threshold temperature would be required to induce panting.

SUMMARY AND CONCLUSIONS

Trained dogs, after a two and a half hour rest, have been given measured dosages of diathermy heat in experiments in which 1, the environmental temperature was kept constant and the rate of heating varied from 0.5 to 1.5 b.m.r. units, and 2, the environmental temperature was varied from 23° to 28°, and the rate of heating maintained at 1.0 b.m.r. There is no one rectal or skin temperature threshold value but the values do fall in a narrow range for any one dog, and are not changed by the rate of temperature change. A low environmental temperature lowers the basal skin temperature and the skin panting threshold temperature. The more obese the dog the lower its skin basal and skin panting threshold temperature. The evidence seems to indicate that the panting in dogs caused by diathermy heat is a peripheral rather than central panting, although the latter is not entirely precluded.

Before panting starts there is a rapid rise in ear temperature to a high value. Since other skin areas show a sluggish temperature response to diathermy heat, the importance of the ears as temperature regulators against heat is emphasized.

In conclusion, I want to express my most sincere appreciation to Dr. H. G. Barbour for much helpful assistance and useful criticism.

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SEASONAL VARIATIONS IN THE NORMAL POLYNUCLEAR COUNT IN MAN

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Recent studies on the normal polynuclear count in man show that the original "healthy" count laid down by Cooke and Ponder (1927) applies only to certain localities, and that marked variations exist, depending on geographical location. MacLeod (1935) has studied blood films obtained from different parts of the world, and has concluded that definite differences exist between the polynuclear means of various localities, though the mean for each locality is quite constant. In reviewing the literature, MacLeod has also pointed out that there are differences in the average polynuclear counts of certain animals, the variations apparently depending either on the genetic strain or on the locality.

Several papers have recently appeared which confirm the existence of these local variations, and show that the polynuclear count in health is considerably more labile than was once thought. Kennedy and Mackay (1935) examined 134 British airmen who had been stationed in Baghdad, Iraq, for some time, and found that their polynuclear counts showed a marked shift to the left (mean 1.99) compared to those of Great Britain (mean 2.66); in fact, their average count was virtually the same as that of the native Iraqi. Bernard Shaw (1936) demonstrated a similar shift to the left in an Egyptian population and showed, furthermore, that prolonged residence either in Egypt or in Great Britain caused a shift in the polynuclear mean, the British mean moving to the left in Egypt and the Egyptian to the right in Great Britain. Pai (1935), in confirming the work of Shaw, studied the polynuclear means of native Chinese before they left for an indefinite stay in England, and found that after six months in the latter country their counts had moved to the right from the original mean (2.00) to that of Britain (2.60). Since all the above workers used the Cooke and Ponder criterion in making the counts, there can be no question as to the reality of their results. They all conclude that environment is responsible for the shifts.

No attempt, however, has been made to determine the stability of the count in a fixed population over a period of time. Since environment is considered important, it is possible that annual variations of climate in

the same locality may exert an influence on the polynuclear count. The following study was made with this idea in mind.

EXPERIMENTAL. The work was done in Cold Spring Harbor upon a population drawn from scientific workers in the laboratories of the Carnegie Institute of Washington and of the Biological Laboratory. The individuals selected ranged in age from 20 to 70 years. Each had been occupied in the same task in the same place for some time, and so their environment was more or less stable. Thirty persons were selected, ten of whom were eliminated during the year because of minor ailments which

TABLE 1

MONTH	I	II	III	IV	V	MEAN	HIGH	LOW	σ	S.E. OF MEAN
September	11.4	48.4	33.8	6.1	0.3	2.36	2.69	1.93	0.19	0.042
October	15.3	52.1	28.8	3.8		2.23	2.61	1.88	0.195	0.043
November	18.2	54.9	24.3	2.6		2.11	2.43	1.67	0.186	0 041
December	28.4	54.9	15.5	1.2		1.89	2.21	1.52	0.178	0.039
January	30.2	53.7	15.3	0.8		1.87	2.22	1.57	0.186	0.041
February	23.4	55.8	19.2	1.4		1.99	2.25	1.74	0.165	0.036
March	19.7	57.4	21.6	1.3		2.04	2.39	1.71	0.198	0.044
April	25.3	54.3	19.4	1.0		1.96	2.28	1.62	0.186	0.041
June	19.7	59.4	19.6	1.3		2.02	2.34	1.72	0.164	0.036
August	10.8	55.8	30.8	2.1		2.24	2.50	1.96	0.140	0.031

TABLE 2

	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	APRIL	JUNE	AUGUST
September		2.5	4.3	7.7	8.3	6.9	5.2	6.9	5.8	2.3
October	2.5		1.7	5.5	5.7	4.1	2.8	4.2	3.0	0.06
November	4.3	1.7		3.8	4.1	2.3	1.1	2.5	1.3	2.5
December	7.7	5.5	3.8		0.03	0.17	2.5	1.2	2.6	7.0
January	8.3	5.7	4.1	0.03		2.0	2.8	1.5	3.1	7.2
February	6.2	4.1	2.3	0.17	2.0		1.1	0.4	1.2	5.4
March	5.2	2.8	1.1	2.5	2.8	1.1		1.0	0.4	3.6
April	6.9	4.2	2.5	1.2	1.5	0.4	1.0		1.5	5.5
June	5.8	3.0	1.3	2.6	3.1	1.2	0.4	1.5		4.2
August	2.3	0.06	2.5	7.0	7.2	5.4	3.6	5.5	4.2	

would be expected to affect the polynuclear count. The results are based upon the counts of 13 males and 7 females.

The blood films were made by the coverglass method, which is greatly preferable to the method of making films on slides. The Jenner-Giemsa staining technique was used throughout, and the count was carried out according to the method of Cooke and Ponder. Differential counts were made at the same time, but they are not being reported upon here; it is enough to say that they were constant and characteristic in each individual.

The results are shown in tabular form. Table 1 gives the average figure in each class for each month, along with the average polynuclear means. The high and low means for each month, the standard deviation of the scatter, and the standard error of the mean are also given. Table 2 shows the number of times the S. E. of the differences between pairs of means exceeds its own standard error.

DISCUSSION. It will be seen that the second count, taken in October, shows a significant shift to the left compared to that for September. This shift continues during the fall and winter months, reaching the lowest mean in January, at which time and during the spring months a slow shift to the right is apparent. Only in the following August does the mean approach significantly the high mean of the previous September.[1]

Examination of the cell classes from month to month shows that class II is the pivotal class throughout, the number in this class remaining fairly steady. The shift is most apparent in class I, which gains at the expense of classes III and IV. Class I is the "Staff" or "non-segmented form," upon which most significance is placed by clinicians. Medlar (1931–32), in fact, considers that a persistence of more than 8 per cent unsegmented forms is abnormal, while Cooke and Ponder place the upper limit of normal at 12 per cent. In this study, the lowest number in class I is 11 (Sept.) and the highest is 30 (Jan.), both of which would be considered abnormal by most authorities. It should be emphasized that none of the twenty subjects complained of any illness during the year, though it is possible that low-grade infections were present in some without occasioning any measurable discomfort. It is more probable that environmental changes produce a physiological adjustment in the body, one manifestation of which is increased bone marrow activity for, without exception, *all of the subjects in this study showed a significant shift to the left in the polynuclear mean.*

Kennedy and Cameron (1935) have suggested that variations in intensity of ultra-violet irradiation may be responsible for the well-established local variation in the count. Before accepting this idea too readily, it should be pointed out that table 1 shows that the count begins to move to the right in February, many months before the subjects were occupied with summer activities and were exposed to ultra-violet irradiation in large amounts, for climate in Cold Spring Harbor during March and April is inclement and not conducive to outdoor activity.

SUMMARY

In a series of 20 normal persons in one locality (Cold Spring Harbor) a variation in the polynuclear count was found throughout the year in

[1] The study was discontinued in August 1937 because too many of the subjects left Cold Spring Harbor.

each of the subjects studied. The highest count (mean 2.36) was found in September and the lowest (1.87) in January. The polynuclear count is therefore subject to seasonal as well as local variations.

I am indebted to Dr. H. A. Charipper of New York University for the facilities of his laboratory and for his helpful advice. This work formed part of a thesis accepted for the Master's degree at New York University. I also wish to thank the subjects of this study for their kindness and patience during the course of the work.

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CARBOHYDRATE CHANGES IN VARIOUS ANIMALS FOLLOWING POTASSIUM ADMINISTRATION[1]

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Recent work has shown that in the rat potassium injection greatly influences blood sugar and tissue glycogen levels (Silvette and Britton, 1937). These experiments have now been amplified, and other animals, the cat and opossum, have been included in the study. The possibility that carbohydrate disturbances in adrenal insufficiency may follow on primary defects in potassium balance gives added interest to the results.

Normal animals fasted for 18 hours but allowed water *ad lib.* were used in all experiments. Both male and female rats, weighing between 80 and 120 grams, were used. Mature cats of both sexes were employed, but only full-grown male opossums. Injections were made intraperitoneally on the basis of body weight of the animal, i.e., 10 cc. of solution per 100 grams. The potassium content of the injected solutions was calculated on the basis of the K ion, i.e., a solution containing 0.1 per cent of potassium actually consisted of 0.25 per cent potassium acetate or of 0.19 per cent potassium chloride. The chemical methods employed have recently been described (Britton, Silvette and Kline, 1938).

RESULTS. *Rats.* Groups of rats injected with various solutions were utilized serially, and their blood sugar and liver and muscle glycogen levels plotted over a $3\frac{3}{4}$ hour period (table 1 and fig. 1). Injection of 5 per cent glucose in normal saline resulted in a large amount of liver glycogen deposition, and there were also increases in muscle glycogen and blood sugar levels. When 0.4 per cent potassium (i.e., 1 per cent potassium acetate) was added to the injected solution, the blood sugar level quickly rose over 100 per cent, while both liver and muscle glycogen were reduced. The addition of only half this quantity of potassium (0.2 per cent), however, brought about no significant change in the various carbohydrate levels.

Thus the administration of potassium along with glucose led to glycogenolysis and hyperglycemia. The same result was obtained when, in place

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of glucose-injected rats, fully-fed rats (with full reserves of tissue glycogen) were used. In these cases the injected solution contained only 0.1 per cent potassium, and 5 per cent urea was used in place of glucose to maintain osmotic pressure. Table 2 shows that at the time of utilization, $2\frac{1}{2}$ hours after injection, the blood sugar of the potassium-injected group was over 20 per cent higher, and the liver glycogen about 40 per cent lower than the controls. Muscle and heart glycogen values were also reduced after potassium injection. It should be emphasized that all the animals in the

TABLE 1

Effect of potassium injections on blood sugar and glycogen of rats

SOLUTION CONTAINING 0.9 PER CENT NaCl, 5 PER CENT GLUCOSE AND	ANIMALS KILLED AFTER INJECTION	AVERAGE BLOOD SUGAR	AVERAGE LIVER GLYCOGEN	AVERAGE MUSCLE GLYCOGEN
	hours	*mgm. per cent*	*per cent*	*per cent*
No K	0.75	123	3.40	0.44
	1.5	116	3.29	0.52
	2.25	132	3.38	0.59
	3.	110	2.39	0.47
	3.75	117	3.64	0.44
0.2 per cent K	0.75	122	4.04	0.50
	1.5	117	2.65	0.46
	2.25	131	1.52	0.43
	3.	105	2.37	0.40
	3.75	98	2.10	0.41
0.4 per cent K	0.75	144	0.37	0.35
	1.5	222	0.59	0.38
	2.25	141	0.88	0.34
	3.	146	0.83	0.34
	3.75	102	0.74	0.39

Animals given 10 cc. solution per 100 grams body weight. Each glucose and glycogen figure the average of 3 experiments.

above series were in good condition when sacrificed, and showed no signs of potassium poisoning.

Cats. The cat was found to be more sensitive than the rat to injection of potassium salts. Administration of 400 mgm. per kilo of potassium (as the acetate or the chloride) was sometimes fatal, but 300 mgm. (or 100 cc. of 0.3 per cent solution) were well tolerated and the animals showed no ill effects. The cats were given solutions containing glucose and sodium chloride with or without potassium, and the blood sugar levels then determined half-hourly. At the end of four hours, several of the animals were used for glycogen determinations. The blood sugar curves showed the same pattern as those described for potassium-injected and control rats,

i.e., hyperglycemia followed the injection of both 0.15 and 0.3 per cent potassium (table 3 A, and fig. 2).

Though terminal glycogen determinations were not made in every case, it was evident from the results that in the cat as well as the rat glyco-

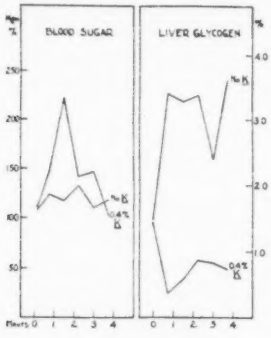

Fig. 1

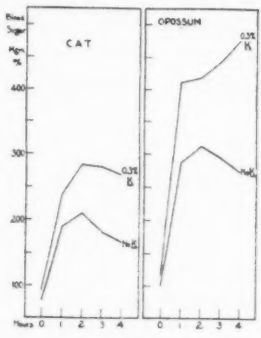

Fig. 2

Fig. 1. Effect of injected potassium on the blood sugar and liver glycogen of rats. Each curve derived from 15 animals.

Fig. 2. Glucose tolerance curves in the cat and opossum after injection of potassium. Each curve derived from 4 or more animals.

TABLE 2

Carbohydrate levels in the rat following injection of potassium

ANIMAL NUMBER	SOLUTION INJECTED	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	CARDIAC GLYCOGEN
		mgm. per cent	*per cent*	*per cent*	*per cent*
1	10 cc. per 100 grams of 5 per	128	1.70	0.30	0.46
2	cent urea plus 0.9 per cent	123	2.70	0.54	0.69
3	NaCl	134	2.86	0.39	0.60
4		127	2.98	0.33	0.83
	Average	128	2.56	0.39	0.65
5	10 cc. per 100 grams of 5 per	159	1.75	0.33	0.53
6	cent urea plus 0.8 per cent	123	1.91	0.38	0.55
7	NaCl plus 0.1 per cent po-	173	1.98	0.31	0.54
8	tassium	171	0.71	0.22	0.52
	Average	159	1.59	0.31	0.54

Samples taken 2.5 hours after injection.

genolysis resulted from the injection of potassium salts. Several serum potassium determinations which were carried out at the end of the experiments showed considerable variation, but the levels four hours after injection were not significantly different in either the potassium-injected or the

control series. It may be mentioned also that in the experiments on rats, terminal serum potassium determinations did not vary significantly between the potassium-injected and control series.

Opossums. The opossum was still more sensitive to potassium salts than the cat. Injection of 100 cc. per kilo of 0.3 per cent potassium was followed sometimes by toxic symptoms, and this was reflected in the marked hyperglycemia in the potassium-injected series when compared to the controls (table 3 B, and fig. 2). It was observed in both the opossum and cat that any sign of potassium poisoning was accompanied by hemoconcentration, which increased progressively as the toxic symptoms developed.

TABLE 3

Blood glucose changes in cats and opossums following injection of potassium acetate solutions

NUMBER IN SERIES	INJECTED SOLUTION CONTAINED 5 PER CENT GLUCOSE AND		AVERAGE BLOOD SUGAR BEFORE INJECTION	AVERAGE BLOOD SUGAR AFTER INJECTION			
	NaCl	K		1 hour	2 hours	3 hours	4 hours
			Cats				
	per cent	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
4	0.9	0	77	190	210	182	167
3	0.7	0.15	88	234	257	247	239
4	0.6	0.3	91	238	283	280	268
			Opossums				
9	0.9	0	100	288	312	295	273
6	0.6	0.3	117	411	419	444	477

Animals given 100 cc. solution per kilo body weight.

No glycogen determinations were carried out on opossums; it seemed undesirable to sacrifice animals when the blood sugar results were so unequivocal. Since in the opossum potassium injection was followed by marked hyperglycemia, it may reasonably be concluded that (in the experimental period allowed) glycogenolysis also took place in these animals.

Discussion. Kylin and Engel (1925) found that the intravenous injection of potassium chloride in man was followed by a fall in blood sugar which, however, rose to normal levels within 30 to 60 minutes. Semler (1925) observed that the administration of potassium chloride to diabetics was usually followed within 2 hours by a rise in blood sugar. Our experiments, carried out over longer periods of time on three animal types and under several different experimental conditions, demonstrate that subtoxic (or non-symptom-producing) doses of potassium salts lead to marked and prolonged hyperglycemia.

In 1922, Myers stated that "there are many observations which lead one to believe that glycogen, creatine, phosphoric acid and potassium are associated in active muscle." Our determinations of muscle glycogen content after potassium injection confirm the existence of at least one of these relationships. Furthermore, cardiac and liver glycogen levels are also depressed by potassium injections, and it is probable that the action of increased potassium salts on tissue glycogen is directly responsible for the hyperglycemia observed.

It may be stated that the intraperitoneal injection of large amounts of glucose solution produces a serious sodium and chloride deficit within the body (Gilman, 1934; Silvette and Britton, 1935; Darrow and Yannet, 1935). In the experiments herein reported, however, all injected solutions were made up with sufficient sodium chloride to maintain serum sodium chloride concentration at normal or even slightly increased levels.

Although potassium determinations were made terminally and not serially during the course of the experiments, it may be concluded from the absence of symptoms and the relatively low terminal values that serum potassium levels were maintained in subtoxic concentration. Glycogenolysis and the resultant hyperglycemia observed after potassium injections could not be considered, therefore, as the result of potassium poisoning. There is suggested, rather, a definite relationship between the potassium ion, or its concentration in blood and tissues, and carbohydrate metabolism.

It is interesting to note that in the plant kingdom, too, potassium concentration and carbohydrate levels are apparently related. White (1936) has indeed suggested that the main effect of the potassium content of plants is the regulation of carbohydrate metabolism by control of the starch-sugar balance. Further reference to this subject has already been made (Britton, Silvette and Kline, 1938).

Possibly only a slight increase in serum (and tissue) potassium concentration may be necessary to bring about exaggerated glycogenolysis, or to inhibit glycogen deposition in the tissues. Thus, amplifying a recently devised scheme of the effects of adrenal insufficiency on the organism (Britton, Silvette and Kline, 1938), it is possible that even the small increases in serum potassium which occur after adrenalectomy[3] may be related to glycogenetic failure, and hence to hypoglycemia. Nevertheless, it must not be forgotten that the relationship between potassium and carbohydrates may be the converse: i.e., that a decrease in cell glycogen may result in release into the blood stream of part of the intracellular potassium content.

[3] According to Truszkowski and Zwemer (1936), the maximum blood potassium level in the cat in potassium poisoning is 70 mgm. per cent. and in adrenal insufficiency it is usually less than 30 mgm. per cent.

SUMMARY

The injection of subtoxic amounts of potassium salts into rats results in a marked rise in blood sugar and decreases in liver, muscle and cardiac glycogen. When a potassium-glucose solution is injected, the blood sugar levels are raised 100 per cent over the control values; but at the same time the marked deposition of liver and muscle glycogen, which occurs on administration of glucose solution alone, is suppressed when potassium is added to the injected solution.

Both cats and opossums given glucose-potassium solutions also show marked and prolonged hyperglycemia compared with potassium-free controls. Liver and muscle glycogen changes (cat) are similar to those observed in the rat.

It appears that an important effect of raising the potassium in the body (as by injection) is the production of glycogenolysis or the inhibition of glycogen formation, and a concomitant hyperglycemia. In the case of adrenal insufficiency, when serum potassium is also increased, there may be a converse relationship: an increased blood and cell potassium concentration may lead to glycogen disappearance; or, the glycogenetic failure after adrenalectomy may result in a release of cell potassium into the blood.

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EXCITABILITY OF THE HYPOTHALAMUS AFTER DEGENERATION OF CORTICIFUGAL CONNECTIONS FROM THE FRONTAL LOBES[1]

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A number of investigations have demonstrated the independent activity of intrinsic hypothalamic neurons in the absence of influences from the cerebral cortex (Bard and Rioch, 1937; Karplus and Kreidl, 1910; Ectors, 1937; and Morrison and Rioch, 1937). This report presents additional evidence of the excitability of the hypothalamic region in the cat after degeneration of corticifugal connections from the frontal lobes.

The preoptic region and hypothalamus of each of 6 cats was electrically stimulated 4 to 6 weeks after bilateral removal of the frontal lobes of the cerebral hemispheres. A description and photographs of the area of ablation have been presented elsewhere with a report of the behavior of the animals (Magoun and Ranson, 1938). The stimulation experiments were performed under Nembutal anesthesia (20 to 25 mgm. per kilo) with the aid of the Horsley-Clarke apparatus in the manner described by Ranson, Kabat and Magoun (1935).

Parasympathetic activity. Contractions of the urinary bladder (fig. 1, upper left) were regularly elicited and appeared just as marked as in the normal animal. They were obtained from the area around the crossing of the anterior commissure, from the preoptic region and from the hypothalamus.

Falls in arterial pressure were not obtained from any of the animals during stimulation, but were occasionally encountered following the cessation of stimulus (fig. 1, lower left). This post-stimulatory effect, which possibly represented a rebound, was elicited from the area around the crossing of the anterior commissure, the preoptic region and the hypothalamus back to about the level of the infundibulum. We are unable to say whether it represented an inhibition of vasoconstrictor or an augmentation of vasodilator activity.

Sympathetic activity. Marked vasopressor responses were regularly elicited from the anteroposterior extent of the hypothalamus and many of the reactions were prolonged as a slowly declining hypertension lasting

[1] Aided by a grant from the Rockefeller Foundation.

for some time after the cessation of stimulus (fig. 1, lower right). Marked dilatation of the pupils and retraction of the nictitating membranes were obtained from the anteroposterior extent of the hypothalamus.

Respiratory activity. Slight decreases in the amplitude of chest movements (fig. 1, upper middle) sometimes associated with slight decreases in respiratory rate, were obtained from stimulation of the area around the crossing of the anterior commissure, the preoptic region and the extent

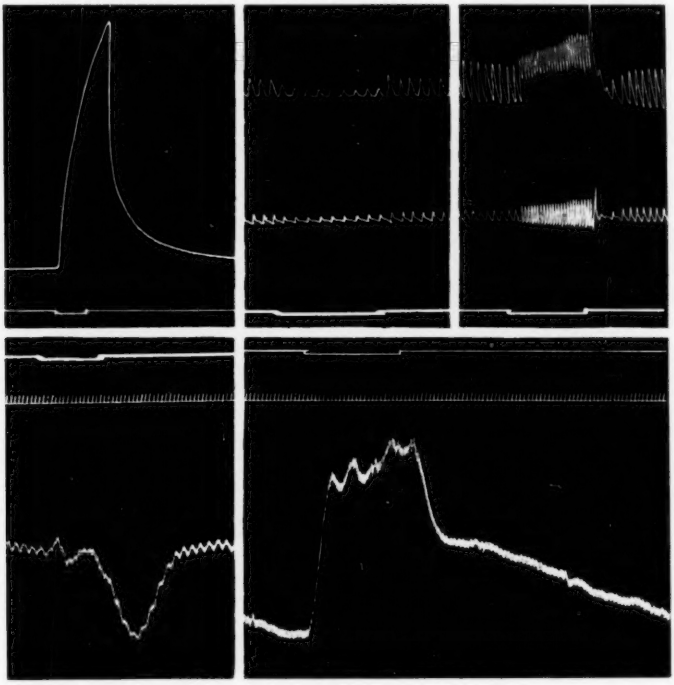

Fig. 1. Vesical (upper left), respiratory (upper right) and vasomotor (lower left and right) responses induced by hypothalamic stimulation in the absence of corticifugal connections from the frontal lobes. The dip in the signal marks the period of stimulation; the time is in second intervals.

of the dorsal hypothalamus. These reactions were much weaker than those which can be obtained in the normal animal.

Marked increases in respiratory rate (fig. 1, upper right) often associated with increases in amplitude of the chest movements were regularly obtained from the anteroposterior extent of the hypothalamus. The facio-vocal activity described as "spitting" was elicited from the anterior hypothalamus as in the intact animal.

Comment. The respiratory inhibitory and vasodepressor responses elicited from the area around the anterior commissure and the preoptic region in these experiments were markedly depressed as compared with responses from normal animals with intact brains (Ranson, Kabat and Magoun, 1935). It does not seem possible to account for this impairment by direct injury to the region as a result of proximity to the cortical lesions, for good contractions of the urinary bladder were obtained from this territory in the same animals. In our experiments on normal animals with intact brains contractions of the bladder (Kabat, Magoun and Ranson, 1936) were not obtained from regions farther forward than the septum and preoptic area, while respiratory inhibition (Kabat, 1936) and vasodepressor responses (Kabat, Magoun and Ranson, 1935) could be traced far forward in the cerebral hemispheres. It was concluded that the representation of the urinary bladder in the basal forebrain was resident in a subcortical collection of neurons, while the respiratory inhibition and vasodepressor responses were dependent, in part at least, upon the activation of descending pathways from the cerebral cortex. The results from the present experiments support this conclusion.

The sympathetic and respiratory excitatory activity induced by stimulation of the hypothalamus in these experiments did not appear to be affected in any way by the absence of connections from the frontal cortex.

SUMMARY

A study of the excitability of the hypothalamic region of the brain of the cat following bilateral degeneration of corticifugal connections from the frontal lobes, showed a considerable loss of excitability for vasodepressor and respiratory inhibitory effects. Contractions of the urinary bladder, pupillary dilatation, retraction of the nictitating membrane, vasopressor responses and respiratory excitatory effects, including facio-vocal activity, were elicited just as in animals with intact brains.

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THE CALORIGENIC ACTION OF AMINO ACIDS IN THE HYPOPHYSECTOMIZED ANIMAL

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A survey of the literature reveals a very evident lack of agreement as to whether the calorigenic action of amino acids is dependent for its manifestation on normal functioning of the hypophysis. It is true that the abundant clinical material studed by Fulton and Cushing (1) and the observations of Johnston (2) indicate that the calorigenic response to protein in cases of hypophyseal insufficiency is of normal magnitude. So also the rather limited experiments of Artundo (3) and Gaebler (4) on hypophysectomized animals would seem to suggest that the phenomenon of calorigenesis by amino acids is independent of the hypophysis. Opposed to these observations, however, are the clear cut experiments of Foster and Smith (5) which demonstrate, so it would seem, that the hypophysis is intimately involved.

In connection with other experiments on amino acid metabolism[1] it seemed to us opportune to re-open the investigation and to study, in particular, the behavior of glycine in the hypophysectomized rat. If we may anticipate the conclusions of this research, described *in extenso* in the ensuing paragraphs, it seems permissible to state that the hypophysis is not concerned with the phenomenon of calorigenesis by amino acids; results to the contrary may perhaps be explained by the fact that the degree of response is intimately related to the mode of amino acid administration and, to a lesser extent, to the quantity given. This does not deny the accepted fact that the basal level of metabolism in the hypophysectomized animal is remarkably low, but this in turn bears no relation to the question of stimulation by amino acids.

With the exception of certain minor changes, the apparatus, experimental procedure, and method of calculation of the results were identical with those described in earlier papers by Lewis and Luck (6).

Modifications in apparatus. A lubricant of light paraffin oil was introduced into the pumps to facilitate their operation. While experimenting with this and other light lubricants, certain toxic symptoms were reg-

[1] By J. M. L. and H. C. S.

ularly observed. The animals appeared cyanotic, and death was commonly preceded by quite regular muscular spasms. Death occurred either during the experiment or within a few days. In those cases in which the animals died in the apparatus there developed a steady decline in the rate of oxygen consumption, accompanied by a marked retention of carbon dioxide. These symptoms have been entirely prevented by filtering the air through a one-inch layer of granulated medicinal charcoal. The filters were introduced into the air lines immediately preceding the entrances into the animal chambers.

The activity of the animals was found to be lessened by illumination of the cages. One hundred-watt lamps were suspended 12 inches above the chambers. The heat produced by radiation was removed by the water in the thermostat.

The movements of hypophysectomized rats are executed more slowly than are those of normal animals. Also, the animals weigh considerably less than do normals. Consequently, the resultant momentum produced during activity is relatively small, and the activity records must be correspondingly more sensitive in order to record the movements. To accomplish this end, two changes have been introduced: the weight, and hence the inertia, of the metal cage has been decreased by using thin aluminum strips for its construction. This has diminished the weight from 385 grams to 85 grams. Also, the cage is now suspended by rubber bands instead of the steel springs which were formerly used. With small increases in tension rubber bands stretch through such relatively great distances that the sensitivity of such an arrangement is not appreciably affected by changes in the position of the cage. With this arrangement it has been found much easier to secure the proper relations of tension on the rubber bands, position of the cage with reference to the rubber balloon, and the degree of inflation of the balloon and tambour to give maximum sensitivity.

Modifications in experimental procedure. It was soon found that the 36 hour fast prior to the experiments led, in the case of hypophysectomized rats, to a high rate of mortality. Consequently, a 12 hour fast has been used throughout, although results of such experiments upon normal animals have been admittedly less consistent than those obtained by Lewis and Luck (6) with the 36 hour fast.

For reasons which will appear in the discussion of the water and saline controls, water was thought to be preferable as a solvent for the amino acids. Therefore, water alone has been used as the solvent in all injections reported in this paper.

Injections were made both orally and intraperitoneally. In both cases the solutions were warmed to approximately 37°C. before injection. The measurements were continued during approximately eight hours after

injection in all cases except in the absence of a metabolic response, in which case they were commonly terminated at approximately four hours. In the event of a response lasting for a period shorter than eight hours, they were terminated soon after return to the basal level provided the four-hour interval had been passed. A few experiments were terminated, for one reason or another, before the end of the eight-hour interval and before return to the pre-injection level. The extra-basal heat production in these was of course less than would have been obtained by continuing for eight hours.

Animals. The experimental animals were hypophysectomized at the age of three months. After hypophysectomy at least one month was allowed to elapse before use in experiments. The ages of the experimental animals averaged 6.2 months (3.8 to 8.0 with the exception of four animals of which the ages were 8.8, 9.0, 14.0, and 15.5, respectively). Their weights averaged 154 grams (94 to 204). The ages of the normal rats used for controls averaged 5.1 months (2.5 to 10), and their weights 220 grams (142 to 334); of these, 4 animals weighed more than 300 grams.

The diet received by the hypophysectomized rats was that upon which they had been raised prior to hypophysectomy. It is designated as "Diet 14" in the Institute of Experimental Biology, University of California. It consists of: whole wheat flour, 67 per cent; fish oil, 5 per cent; casein, 5 per cent; alfalfa meal, 10 per cent; fish meal, 10 per cent; salt, 3 per cent.

During fourteen of the later experiments of the series daily injections of 1 cc. of 20 per cent glucose were given for the purpose of improving the nutritional state of the animals. With one exception, no irregularities were observed in these cases. It seems highly improbable that this single exception, the negative response after oral injection of 2.66 m*M* of glycine, is to be explained by the daily injections of glucose.

The normal animals received the regular stock diet which is in use in the laboratory at Stanford University:

Cracked wheat	2.6 kilos	Alfalfa meal	500.0 gms.
Oatmeal	2.6 kilos	Yeast powder	200.0 gms.
Corn meal	2.6 kilos	Bone meal	150.0 gms.
Flaxseed meal	1.0 kilo	Sodium chloride	50.0 gms.
Milk powder	1.0 kilo	Cod liver oil	150.0 gms.

Number of experiments. Inclusive of controls, 40 experiments with normal rats and 41 with hypophysectomized rats are here reported. In addition, 52 others were run but have been excluded from this report because of excessive activity of the animals, resulting in uncertainty of the metabolic levels.

Basal metabolic rates. From experiments reported in this paper the basal metabolic rates of the normal rats in terms of Calories[2] per day per

[2] 1 Calorie = 1 kilocalorie = 1000 calories.

square meter of body surface averaged 783, with an average deviation from the mean of 51. The average for the hypophysectomized rats was 447, with an average deviation from the mean of 34.

Experiments. Oral administrations of 1.33 m*M* of glycine to hypophysectomized rats gave quite consistent increases, averaging +0.46 Calorie. This value lies 0.25 Calorie above the average for water controls with hypophysectomized rats (see fig. 1). Four experiments with 2.66 m*M* given orally to hypophysectomized rats gave increases of approximately equal magnitude, averaging +0.58, or 0.37 Calorie above the average for water controls. The fifth experiment gave a marked

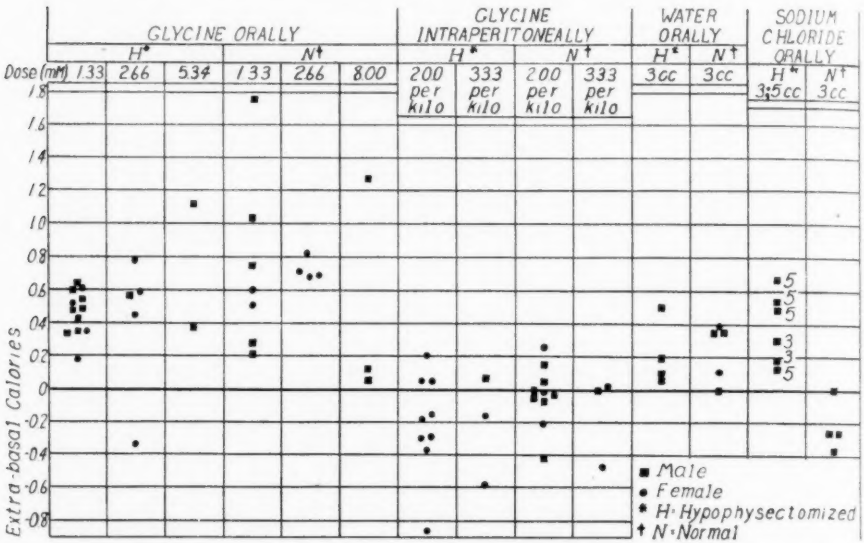


Fig. 1

decrease, which as yet remains unexplained. Two experiments with 5.34 m*M* given orally gave definite increases, the mean being +0.74, i.e. 0.53 Calorie above the water-control average.

In order to duplicate experiments reported by Foster and Smith doses of glycine of 20 m*M* per kilogram of body weight were given intraperitoneally to nine female hypophysectomized rats. The average of all nine experiments amounted to −0.21 Calorie. Larger doses of 33.35 m*M* per kilo of body weight given intraperitoneally also gave decreases, averaging −0.22 Calorie, showing that this is not merely a case of increased threshold of dose for stimulation. It will be noted that these results are in fair agreement with those of Foster and Smith.

Controls. In order to determine how much of the increase obtained after oral injection was caused by the glycine itself, control experiments with oral injections of 3 cc. of water to hypophysectomized rats were conducted. To determine in what way and to what extent the experimental results were affected by removal of the hypophysis, a number of control experiments were run upon normal animals; these included varying doses of glycine given both orally and intraperitoneally. Also, to aid in the interpretation of these controls upon normals, oral injections of 3 cc. of water were given to normal rats.

The water controls with hypophysectomized rats resulted in an extra-basal heat production averaging +0.21 Calorie. Therefore, only heat-production values above this average have been considered due to amino acid stimulation.

Three cubic centimeters of water given orally to normal rats gave increases averaging +0.24 Calorie. This value, it will be noted, is only slightly greater than that obtained with hypophysectomized rats.

Controls in which 1.33 m*M* doses of glycine were given orally to normal rats gave a rather wide distribution of values, averaging +0.77 Calorie, 0.53 above the water-control average. Since 5 of these 11 runs were discontinued at approximately six hours after injection and in 3 of these 5 the effects were incomplete, being still above the basal, this average is probably below the value which would have been obtained if these 3 had been continued for eight hours. With 2.66 m*M* of glycine the results were quite consistent, averaging +0.75 Calorie, i.e., 0.51 above the water-control average. The average magnitude of response did not increase appreciably with the larger dose. One experiment with 8.00 m*M* is reported in which the effect was increased over that of the smaller doses, but not in proportion to the increased magnitude of the dose. Two additional experiments of similar nature gave only small increases in heat production; their validity must be discounted, however, because of the abnormally high pre-injection levels, namely, 890 and 907 Calories per day per square meter of body surface. It would appear from these and other observations we have made that the usual metabolic increases cannot be obtained over abnormally high basal metabolic rates.

Intraperitoneal injections of glycine to normal rats gave responses averaging −0.03 Calorie with the 20 m*M* per kilo dose and −0.15 Calorie for the 33.35 m*M* per kilo dose. These results are similar to those obtained with hypophysectomized rats after intraperitoneal injections.

As a final series of controls, sodium chloride solutions of 0.9 per cent were administered in 3 or 5 cc. portions, the object being to determine whether the calorigenic property of glycine might be common to other simple electrolytes. The results were quite unexpected in that the sodium chloride depressed the metabolic rate of the normal animal and elevated

that of the hypophysectomized. The increase in the latter case (six animals) averaged 0.40 Calorie. Compared with the metabolic effect in normal animals receiving sodium chloride the true increase was more nearly 0.60 Calorie.

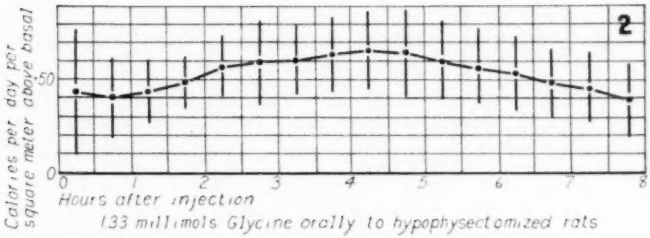
It occurs to us that the following explanation may be reasonably proposed. The very low basal metabolic rate of the hypophysectomized animal is probably due not only to decreased thyroid function but to decreased function of the adrenal cortex also. In Addison's disease, in which there is extensive degeneration of the adrenal cortex, sodium chloride administrations are known to effect a marked improvement. It may be expected, therefore, that sodium chloride administered to an animal suffering from the adreno-cortical deficiency of hypophysectomy would also improve its general well-being and elevate somewhat its otherwise low metabolic rate.

Respiratory quotients. The effect of hypophysectomy upon the respiratory quotient was determined incidentally to the other observations recorded here. In 59 normal rats (41 males, 18 females) fasted for 12 hours, the respiratory quotient averaged 0.745 with an average deviation from the mean of 0.02. Twenty-two normal males fasted for 36 hours gave a respiratory quotient of 0.725 ± 0.02. In 41 hypophysectomized animals (24 males, 17 females) fasted for 12 hours the respiratory quotient averaged 0.82 with an average deviation from the mean of 0.04. Twelve hypophysectomized males fasted for 36 hours gave a respiratory quotient of 0.81 ± 0.05.

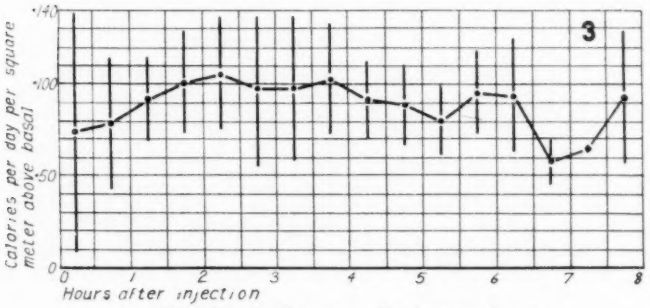
The respiratory quotient of the hypophysectomized animal is, therefore, significantly greater than that of the normal.

Charting of results. To show the general course which the metabolic levels have followed after injection and the degree of uniformity among experiments of similar nature, figures 2 to 9 are presented. They show for each type of experiment the average differences from the pre-injection or basal level in terms of Calories per day per square meter of body surface. These averages have been obtained by dividing individual experiments into half hour periods, calculating the distances from the basal level for each period, and then averaging corresponding periods from the various experiments. The lengths of the vertical lines drawn through the points represent the average deviations from the means.

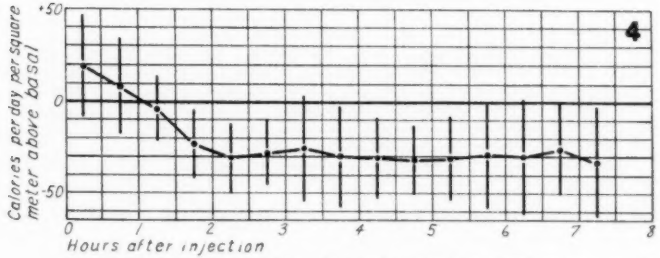
Experiments in which the metabolic rates underwent a change after the injection and then returned in a few hours to the pre-injection level were discontinued soon after the basal level was reached, as described above. It is assumed that they would have been maintained during the remainder of the eight hour period at that level had they been continued longer. Therefore, these additional zero points have been averaged in with the levels from other experiments which were continued for eight hours.



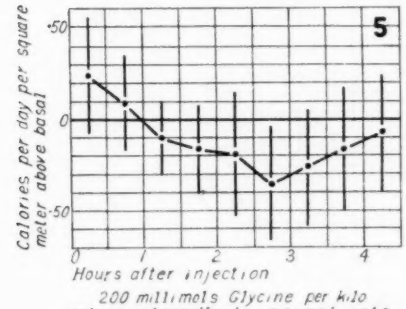
1·33 millimols Glycine orally to hypophysectomized rats



2·66 millimols Glycine orally to normal rats



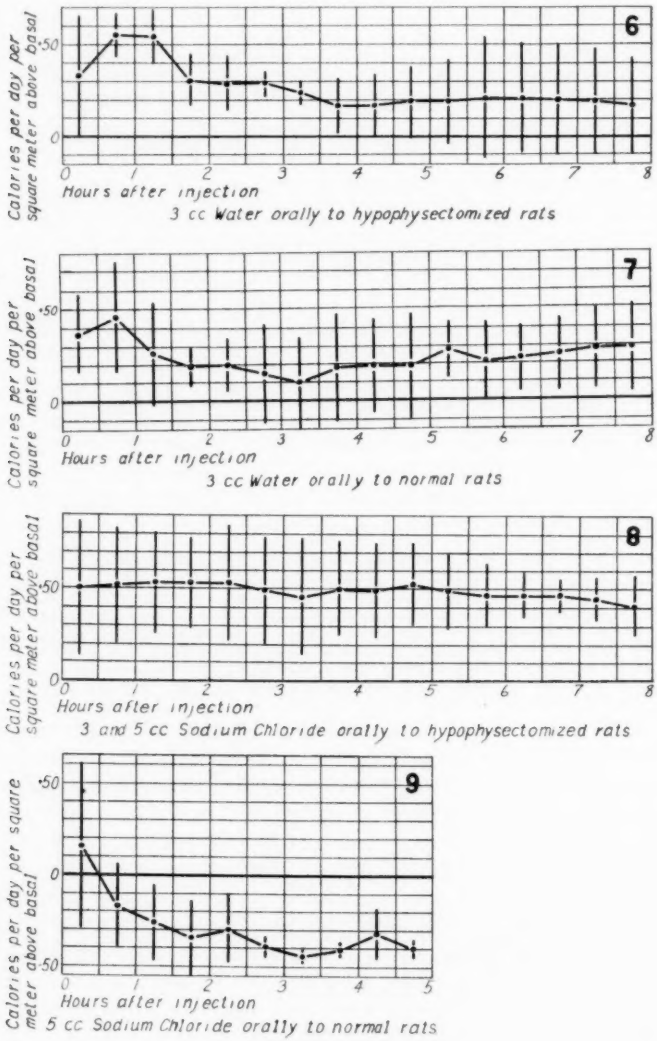
20·0 millimols Glycine per Kilo intraperitoneally to hypophysectomized rats



2·00 millimols Glycine per kilo intraperitoneally to normal rats

Figs. 2 to 5

It will be observed that after the oral injection of glycine to hypophysectomized rats the metabolic level did not return immediately to



Figs. 6 to 9

the basal level, but rather the effect was of long duration. The same was true in the normal animals. In the latter case this was undoubtedly a

result of the relatively short fast employed, since Lewis and Luck (6), with normal rats fasted for 36 hours, found that return to the basal level occurred within two to three hours after injection.

It would appear from these results that after oral injection of glycine an increase in heat production is obtained both in the normal and in the hypophysectomized rat. After intraperitoneal injection no increase but, in fact, a slight decrease is obtained in both types of animals. We conclude, therefore, that the metabolic response following injection of glycine is qualitatively unaffected by removal of the hypophysis.

SUMMARY

1. Hypophysectomy does not abolish the calorigenic action of glycine, orally administered, in rats.

2. Glycine, administered intraperitoneally, is without any calorigenic effect in either the normal or hypophysectomized rat.

3. The metabolic rate of the hypophysectomized rat is 40 to 45 per cent below that of the normal animal.

4. Moderate injections of sodium chloride provoke a prompt increase of 6 to 15 per cent in the metabolic rate of the hypophysectomized animal.

5. The respiratory quotient of the hypophysectomized rat is appreciably greater than that of the normal animal.

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THE RELATION BETWEEN CARBOHYDRATE AND β-HYDROXYBUTYRIC ACID UTILIZATION BY THE HEART-LUNG PREPARATION

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The prevention or cessation of ketosis consequent to the administration of glucose to diabetic or non-diabetic animals may be attributed to either an increased utilization of ketone bodies (ketolysis) or a decreased formation of these substances (antiketogenesis). Although numerous studies indicate glucose to have no ketolytic effect (1–5) some authors continue to write of a "ketolytic" ratio of from one to two.

In order to obtain more crucial data, whether or not such a fixed ketolytic ratio exists, we have studied the utilization of ketone bodies by the heart-lung preparation. This preparation has certain peculiar advantages for such a study. Thus the perfusing blood can be readily deprived of all glucose and available lactate, and one can vary at will the carbohydrate and the ketone concentrations of the perfusate. When the carbohydrate content is depleted, the heart receives no carbohydrate from the circulating blood. Further, the heart appears to use none, or at most, very little, of its glycogen,[1] when perfused with blood containing no sugar and only low concentrations of lactic acid. This is in agreement with the observations of Cruickshank and co-workers (6) that during marked hypoglycemia, the heart does not utilize appreciable amounts of carbohydrate for energy.

If ketone utilization is dependent upon the simultaneous oxidation of carbohydrate, there should be no removal of ketone bodies in the absence of circulating carbohydrate, and when both are present in the blood a definite positive correlation should be found between the removal of carbohydrate and of ketone bodies.

EXPERIMENTAL. The heart-lung preparation was set up in the manner described by Patterson and Starling, one dog being bled and its blood sub-

[1] Unpublished experiments of Waters and Fletcher, in which they perfused heart and lung with sugar-free blood under the same conditions as those of the experiments described in this present paper, yielded results indicating only a slight decrease, of doubtful significance, in the glycogen content of the hearts as compared with that of control hearts. Nor was there any decrease in the small concentration of glycogen in the lung tissue.

sequently used to perfuse the heart and lungs of a second dog. Generally this blood, drawn several hours before the actual experiment, was treated according to the method described by Waters and Fletcher (7) for the removal of glucose. In some experiments this preliminary treatment was not carried out.

When the preparation was completed, β-hydroxybutyric acid was introduced into the circulating blood in the form of its sodium salt. After allowing a fifteen-minute interval for diffusion, a sample of blood was drawn for the determination of blood sugar by the method of Shaffer and Somogyi, of blood lactic acid by the Friedemann, Cotonio and Shaffer method, and of blood ketones by the Van Slyke and Fitz method. These estimations were repeated after one hour and after two hours. In some experiments glucose was added at the end of the first hour period. In

TABLE 1

EXPERIMENT NUMBER	WEIGHT OF HEART	FIRST HOUR			SECOND HOUR		
		A	B	Mols B / Mols A	A	B	Mols B / Mols A
	grams	*mgm.*	*mgm.*		*mgm.*	*mgm.*	
1	53.5	17	69	7.5	0	89	∞
2	50.2	25	50	3.4	31	115	6.5
3	74.2	67	8	0.2	68	24	0.6
4*	60.0	148	49	0.6	189	4	0.0
5†	107.5	119	90	1.3	167	26	0.2
6†	68.3	109	39	0.6	129	42	0.6

A, Total milligrams glucose and lactic acid removed from circulation in 1 hour.
B, Total milligrams β-hydroxybutyric acid removed from circulation in 1 hour.
* High blood sugar to start; one gram malonic acid as sodium malonate added at beginning.
† Glucose added at end of first hour period.

view of the observations that heart glycogen is not readily lost, the total carbohydrate utilized by the system was computed by multiplying the amount of glucose and lactic acid removed from each cubic centimeter of blood, by the total blood volume. The ketone body utilization was calculated in a similar manner.

Six experiments in which two one-hour intervals were studied are summarized in table 1. It may be noted that the molecular ratios of ketones oxidized to glucose oxidized vary from 0.2 to ∞ and that an increase in the rate of ketone utilization is not associated with an increase in carbohydrate utilization. In one experiment, the molecular ratio for the second hour period is practically zero. In this experiment sodium malonate was added to the perfusate, and it may have exerted an inhibitory effect on the uptake of β-hydroxybutyric acid similar to the inhibition noted by Quastel and

TABLE 2

EXPERIMENT NUMBER	WEIGHT OF HEART	FIRST HOUR			SECOND HOUR		
		A		B	A		B
		Glucose	Lactic acid		Glucose	Lactic acid	
	grams	*mgm. in 100 cc.*	*mgm. in 100 cc.*	*mgm. in 100 cc.*	*mgm. in 100 cc.*	*mgm. in 100 cc.*	*mgm. in 100 cc.*
1	53.5	12-2	20-25	52-31	2-0	25-24	31-3
2	50.2	12-0	29-32	160-142	0-0	32-19	142-94
3	74.2	64-43	34-30	33-30	43-11	30-33	30-20
4*	60.0	162-113	23-22	70-53	113-50	22-14	53-52
5†	107.5	27-0	31-17	79-48	71-13	17-11	48-38
6†	68.3	12-0	27-8	86-75	70-27	8-8	75-61

A, Concentrations of glucose and lactic acid in blood at beginning and end of hour periods.

B, Concentrations of β-hydroxybutyric acid in blood at beginning and end of hour periods.

* High blood sugar to start; one gram malonic acid as sodium malonate added at beginning (blood volume = 300 cc.).

† Glucose added at end of first hour period.

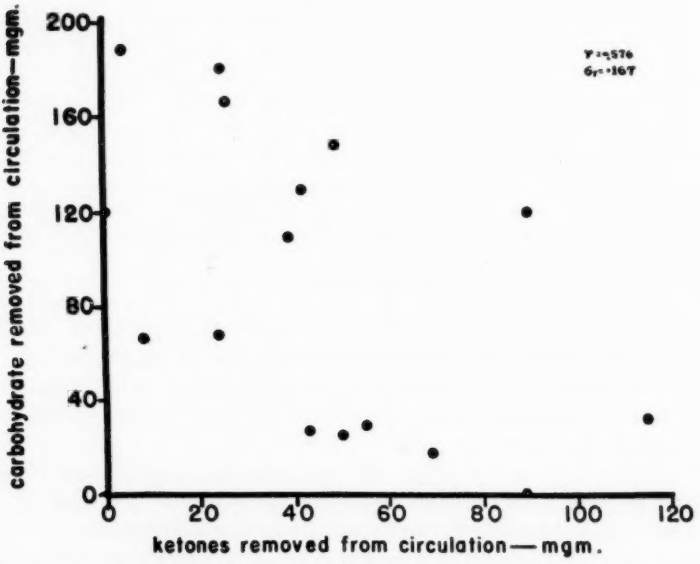

Fig. 1. The relation between carbohydrate and β-hydroxybutyric acid removed from the circulating blood of the heart-lung preparation.

r = the coefficient of correlation (−0.576).

σr = standard error of r (0.167).

Wheatley (8) in the instance of acetoacetic acid in rat kidney slices. Table 2 shows the absolute concentrations of glucose, lactic acid and β-hydroxybutyric acid in all of the above experiments. In order more clearly to illustrate the relationship between carbohydrate and ketone utilization, the values obtained in sixteen one-hour intervals are shown in figure 1.

Discussion. The hypothesis that the oxidation of ketones is dependent upon the simultaneous oxidation of carbohydrate is not supported by our results. If this hypothesis were correct, we should find a positive correlation between sugar and ketone body utilization, whereas our data indicate that, under our experimental conditions, the chances that such a positive correlation would occur are less than 2 in 10,000. There is no semblance of any definite ratio between the utilization of carbohydrate and acetone bodies.

In these experiments the cardiac output and peripheral resistance were maintained fairly constant throughout the periods of observation; with an output of 100 to 120 cc. per minute against an external pressure of about 90 mm. Hg. The rate of utilization of carbohydrate material by the heart from the perfusing blood depends not only on the amount of work performed by the heart and on the concentration of substrate, but also on a number of undefined factors. There is no reason to doubt but that the utilization of acetone bodies is similarly influenced. These facts should be borne in mind when interpreting data from heart-lung experiments.

In experiments 1, 2 and 3 there is an increase in the usage of β-hydroxybutyric acid during the second hour over that of the first, while the carbohydrate usage is lower, due in part at least, to the decreased availability of this material. These results may be compared with those of experiments 5 and 6, in which extra sugar was added at the end of the first hour period. In these latter experiments, in spite of an adequate supply of β-hydroxybutyric acid, there is no increased utilization of this substance in the second hour (in experiment 5 there is a marked decrease), while there is an increased utilization of carbohydrate. These results suggest a preferential utilization of carbohydrate by the heart, when present in such concentrations as to provide an adequate supply.

Statistical analysis of the data illustrated in figure 1 reveals a negative correlation between carbohydrate and ketone utilization, with a coefficient of correlation of -0.576 of which the standard error is 0.167.

The observations that instead of facilitating ketone utilization in the heart-lung preparation, carbohydrate inhibits it, are in accord with the theory of substrate competition for available oxygen. Krebs (9) first proved the existence of substrate competition when he demonstrated that the deamination of l-amino acids in surviving kidney tissue is inhibited readily by oxidizable substrates. Edson (10) subsequently obtained evidence which revealed that fatty acids compete with other oxidizable

substrates for the oxidizing systems of the liver. Our studies with the heart-lung preparation indicate that when both carbohydrate and ketone bodies are available to the heart, the former is used in preference to the latter (see tables 1 and 2). However, this does not necessarily apply to all muscles, since no evidence of substrate competition was obtained in eviscerated animals by Mirsky and Broh-Kahn (5). It is probable that this phenomenon is a characteristic of cardiac musculature, but more evidence is necessary if this is to be established.

It is noteworthy that even when the heart presumably is deriving most, if not all, its energy from the oxidation of fat, it is still able to use appreciable quantities of β-hydroxybutyric acid.

The foregoing observations indicate that glucose is not ketolytic and that therefore its effect in ketosis must be due to an antiketogenic action (fat-sparing). Since the ketone bodies are manufactured chiefly, if not entirely, in the liver, the antiketogenic action of glucose must be due to an inhibition of fat metabolism in that organ (11), i.e., a substitution of fat oxidation by that of carbohydrate, which accords with the recent views on substrate competition in the liver.

SUMMARY AND CONCLUSIONS

There is no positive correlation between the utilization of carbohydrates and that of ketone bodies by the heart-lung preparation. The "ketolytic" ratios obtained varied from 0.2 to ∞.

It is suggested that carbohydrate is used preferentially by the heart, and that when in addition ketone bodies are made available to the cardiac muscle, a competition between the substrates occurs.

The action of glucose in diabetic and non-diabetic ketosis is attributed to an antiketogenic or fat-sparing effect.

We wish to thank Mr. James Campbell for assistance with the acetone body determinations.

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